Coronary artery disease is becoming an increasingly common cause of heart failure, with ≈65% of heart failure patients in the United States having ischemic heart disease.1 Each year, 635,000 people in the United States experience their first myocardial infarction (MI), with another 280,000 having a recurrent MI.2 This results in 125,664 deaths, with 15% of MI patients dying in the first year.2 Although mortality rates for MI are declining, survivors have an increased risk of death from 1.5% to 15% over the general population due in large part to an estimated 24% of MI patients ultimately developing heart failure.2,3 Thus, there is a clear clinical need to prevent myocardial cell death to reduce mortality due to acute MI, but also to improve long-term outcomes by minimizing subsequent development of heart failure. Coronary heart disease as a whole costs the United States $195.2 billion a year, with the cost expected to double by 2030,2 underscoring the need for the discovery of translatable approaches to prevent ischemic cell death.

Ischemia/reperfusion injury causes cell death because of a well-described, albeit very complex, process involving lack of oxygen, calcium overload, disruption of the sarcolemma, ATP depletion, and reactive oxygen species generation.4,5 Almost 3 decades ago, Murry et al6 described the cardioprotective effect of ischemic preconditioning, wherein short bouts of ischemia/reperfusion before a prolonged ischemic episode were shown to reduce subsequent infarct size. This observation has since been confirmed in hundreds of laboratories and extended to multiple mammalian species and other organ systems, demonstrating that the innate protective mechanisms activated by brief ischemia/reperfusion are conserved. Moreover, subsequent investigations have shown that pharmacological agents can induce selective arms of the complex physiological response to brief ischemia/reperfusion, reaping some of the infarct-sparing benefits.7,8 In addition to classical or early preconditioning, which is transient (lasting on the scale of an hour), ischemic and pharmacological preconditioning induces a late phase of protection, which manifests ≈24 hours after the preconditioning stimulus and lasts several days.7 More recently, it was observed that intermittent blood flow during the reperfusion of an index ischemic event could postcondition the myocardium, conferring protection similar to that seen with preconditioning.9,10 Importantly, investigation of the basic phenomena of pre/postconditioning in patients (in the setting of cardiac surgery) has demonstrated these pathways to be operative in humans.11 Together, this evidence is a sound rationale for investigating the mechanisms of ischemic preconditioning as a strategy to identify treatments for acute MI and ischemic heart failure in humans.

A common theme that has emerged from mechanistic studies of ischemic preconditioning and other cardioprotection studies is that pathways involved in proliferation and growth in noncardiac cells tend to induce or participate in protection against ischemic cell death in myocytes. Among many promising candidates fitting this description, the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathway has been convincingly shown to promote cell survival in the heart.12,13 Nuclear targeting of Akt protects against ischemia/reperfusion injury,14 whereas the loss of PHLPP-1, the Akt phosphatase, improves myocardial survival.15 Various preconditioning stimuli activate Akt in the heart,13,16 and its phosphorylation on both Thr308 and Ser473 is required for full activation of the molecule.17 A well-studied regulator of PI3K/Akt signaling is the mechanistic target of rapamycin (mTOR), a serine/threonine kinase originally identified in a mutagenesis screen in yeast. Initial studies found that the yeast genes tor1 and tor2 potentiated the cell cycle inhibitory effects of rapamycin.18 In mammals, FKBP12 binds rapamycin and the two interact with mTORC1 to inhibit it. A hallmark of mTOR is its function in distinct protein complexes, termed mTORC1 and mTORC2, containing proteins Raptor and Rictor, respectively.19 mTORC1 has been shown to sense and integrate growth factor signals along with energy levels to control protein synthesis.19,20 mTORC2 regulates cytoskeleton organization and autophagy.19,20 Importantly, the Rictor-containing mTORC2 carries out rapamycin-insensitive actions of mTOR.21 In this issue of Circulation Research, Yano et al22 convincingly show that mTORC2 is necessary for ischemic preconditioning in a murine perfused heart model. These findings build on a rich body of literature surrounding the Akt pathway, which identified mTORC2 as the main kinase for Ser473 phosphorylation on Akt23 and established the importance of Akt signaling in ischemic preconditioning and other cardioprotective interventions.13 Yano et al conducted a screen for proteins targeted by mTOR/ischemic preconditioning–driven phosphorylation and identified ribosomal protein S6 (Rps6) phosphorylation as a downstream target of ischemic preconditioning signaling. Through an exhaustive set of in vivo experiments, the authors demonstrated that this pathway is necessary for the reduction in infarct size and preservation of cardiac function by ischemic preconditioning, in that treatment...
with mTORC1/2 inhibitors Ku63794 and pp242, but not the mTORC1 inhibitor rapamycin, blocked the effect if administered throughout the preconditioning protocol. It was already known that mTORC1 is activated by Akt and regulates Rps6 phosphorylation via a Rps6 kinase (p70S6K).24,25 An important kinase for ischemic preconditioning.26 The present study revealed a key component of this pathway in ischemic preconditioning that was previously unrecognized: Rps6 phosphorylation increases (despite unchanged levels of Rps6 total protein) after ischemic preconditioning in mice, and the loss of Rps6 is sufficient to exacerbate H2O2-induced cell death and block insulin–induced protection in isolated cells.

Yano et al22 show that during ischemic preconditioning, phosphorylation of Rps6 is critical for potentiating the activity of mTORC2 to phosphorylate Akt, creating a positive feedback loop that requires direct interaction of Rps6 with mTORC2. This finding provides crucial insight into a previous report that mTORC2 can be activated in a translation-independent manner via association with intact ribosomes,27 representing an observation from the current study that is likely to impact the broader field of mTOR biology beyond the cardiac literature. Individual signaling proteins operate in multiple pathways, performing the same function at the atomic level but with very different outcomes for cell biology based on distinct interaction partners. mTOR signaling is a terrific example of this principle: when interacting with Raptor or Rictor, forming mTORC1 or mTORC2, respectively, mTOR activates distinct signaling events. As demonstrated in the current study, further classification of mTORC2 complexes into those bound with ribosomes/Rps6, versus those not, accordingly determines the ability of mTOR to lead to sustained Akt activation (see the Figure). For the novel positive feedback motif of mTORC2/Rps6 to Akt to lead to drug development, future studies will need to work out the direct mechanism by which Rps6 phosphorylation activates mTORC2 and the molecular component of the mTORC2 complex on which it acts. Furthermore, as the authors highlight in red in their schematic (Figure 4 in Yano et al), the subcellular localization of these events is still unclear. It has previously been shown that mTORC2 can phosphorylate a different residue of Akt (Thr450) during Akt translation by associating with ribosomes actively translating Akt mRNA.28 This may not be the case for Ser473 phosphorylation, the residue examined by Yano et al, because previous studies in endothelial cells have shown that mTORC2 recruitment to lipid rafts in the plasma membrane is necessary for Akt activation.29 Additionally, Yano et al show that only the activity of ribosome-bound fraction of mTORC2 is responsive to Rps6 phosphorylation, suggesting that this entire complex of mTORC2 and Rps6 may be somehow interacting with Akt, though this is not measured in the current study in vivo. Finally, Yano et al make the interesting observation that Rps6 phosphorylation acting on mTORC2 has no effect on initial Akt phosphorylation occurring 5 minutes after ischemic preconditioning, but Rps6 phosphorylation is necessary to maintain Akt phosphorylation 15 minutes after ischemic preconditioning. This is an alluring observation because of the transient nature of ischemic preconditioning–induced benefits against subsequent injury; however, it remains unclear whether such a perpetual activation of survival signaling would be possible (or even desirable) as a therapeutic approach.

Overall, the study by Yano et al22 rigorously establishes the in vivo role of mTORC2 in preconditioning and uncovers a novel interaction between mTORC2 and Rps6 that may shed new light on the basic pathways of PI3K/Akt/mTOR signaling. This current work also supports the findings of a very recent study that showed that the loss of Rictor increased cardiomyocyte death in cell culture (potentiating the effect of H2O2) and worsened fibrosis, increased apoptosis, and further impaired cardiac function after MI in the mouse heart.30 These studies highlight the promise of molecular intervention against specific signaling events to achieve a therapeutic benefit, as described31 for other survival kinases in a variety of settings including cardiovascular disease. Clinical implementation of pharmacological or surgical strategies targeting ischemic preconditioning has been thoroughly reviewed,16,32,33 highlighting the current lack of translation, despite substantial mechanistic, animal studies, and in vivo proof of concept in humans. The well-recognized hurdles to translation, including comorbidities, genetic differences, experimental protocol variation, and metrics for therapeutic benefit, led to the creation of the National Institutes of Health–funded Consortium for preclinical ASSEment of CARDioprotective therapies (CAESAR),34 which aims to conduct rigorous, standardized preclinical studies in animal models to evaluate new therapeutic agents for acute MI in a manner similar to a clinical trial.

Figure. Translating mechanistic target of rapamycin (mTOR) signaling for treatment of ischemic heart disease. mTOR exists in the mTORC1 or mTORC2 complex, distinguished by interacting proteins including Raptor and Rictor, with each complex regulating distinct aspects of cell growth (middle panel). In the heart, the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt/mTOR pathway has been previously studied in ischemic preconditioning (PC; as delineated in the top panel). However, the role of mTORC2 remained equivocal. Yano et al22 show that mTORC2 operates in a positive feedback loop necessary for ischemic PC signaling. Ribosomal protein S6 is phosphorylated in ischemic PC, activating a ribosome-bound mTORC2 fraction, which increases the activity of mTORC2 to phosphorylate and activate Akt (novel findings indicated by dashed lines). To promote translating signaling pathways into therapeutics, intermediate cellular phenotypes may be measured as functional outcomes in drug screens (bottom panel). These studies would be complementary to pathway dissection for developing novel drugs. TSC indicates tuberous sclerosis complex.
Such approaches, which study efficacy in larger animals such as rabbits and pigs, will be critical to determine whether novel findings in cells and mice have promise for future translation.

Is comprehensive reconstruction of a molecular pathway the way forward with clinical translation? If so, these studies must be coupled with drug development that screens for specific signaling events, that is, targeting a subset of molecular actions of a given protein in an isoform- and protein complex-specific manner (Figure). Contemporary efforts to manage the debilitating syndrome of heart failure take two fundamentally distinct approaches: recognize that acute MI is unpredictable and that myocyte death will occur, and attempt to treat heart failure through device- or cell-based intervention (fix it once it is broken) versus the aforementioned approach of preventing myocyte death (avoid damage in the first place). Much has been learned about the innate mechanisms of cell survival and that myocyte death will occur, and attempt to treat heart failure through device- or cell-based intervention (fix it once it is broken) versus the aforementioned approach of preventing myocyte death (avoid damage in the first place). Much has been learned about the innate mechanisms of cell survival and that myocyte death will occur, and attempt to treat heart failure through device- or cell-based intervention (fix it once it is broken) versus the aforementioned approach of preventing myocyte death (avoid damage in the first place). Much has been learned about the innate mechanisms of cell survival and that myocyte death will occur, and attempt to treat heart failure through device- or cell-based intervention (fix it once it is broken) versus the aforementioned approach of preventing myocyte death (avoid damage in the first place).

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None.

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Positive Feedback in Cardioprotection: Can More Mechanism Lead to Translation?
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