Cardiac hypertrophy is regulated by a complex interplay of pro- and anti-hypertrophic factors. Here, we report a novel anti-hypertrophic pathway composed of catalase, protein kinase CK2 (CK2), and apoptosis repressor with caspase recruitment domain (ARC). Our results showed that ARC phosphorylation levels, CK2 activity, and catalase expression levels were decreased in the hearts of the angiotensinogen transgenic mice and in cardiomyocytes treated with the hypertrophic stimuli, including phenylephrine, tumor necrosis factor-α, and angiotensin II. To understand the role of ARC in hypertrophy, we observed that enforced expression of ARC could inhibit hypertrophy. Knockdown of endogenous ARC or inhibition of its phosphorylation could sensitize cardiomyocytes to undergoing hypertrophy. The phosphorylatable, but not the nonphosphorylatable, ARC could inhibit hypertrophy. Thus, ARC is able to inhibit hypertrophy in a phosphorylation-dependent manner. In exploring the molecular mechanism by which CK2 activity is reduced, we found that CK2 was carbonylated in angiotensinogen transgenic mice and in cardiomyocytes treated with the hypertrophic stimuli. The decrease in catalase expression led to an elevated level of reactive oxygen species. The latter oxidatively modified CK2, resulting in its carbonylation. CK2 lost its catalytic activity upon carbonylation. ARC is phosphorylated by CK2, and ARC phosphorylation levels were reduced as a consequence of the decrease of CK2 activity. To understand the molecular mechanism by which ARC inhibits hypertrophy, we observed that ARC could inhibit the activation of mitochondrial permeability transition. These results suggest that catalase, CK2, and ARC constitute an anti-hypertrophic pathway in the heart.

Cardiac hypertrophy is a common response to a variety of physiological as well as pathophysiological stimuli. The hypertrophic response enables the heart to meet the increased functional demands. However, sustained cardiac hypertrophy is often accompanied by maladaptive cardiac remodeling leading to heart failure.

Cardiac hypertrophy remains a major dilemma; a growing number of hypertrophic inducers but, as yet, only a few inhibitors have been identified. Apoptosis repressor with caspase recruitment domain (ARC) is involved in apoptosis inhibition (1–6). Its high cardiac expression levels make it a unique and central cardioprotective agent in the heart (1, 7). Although hypertrophy and apoptosis are two distinct cellular events, many stimuli can induce both hypertrophy and apoptosis. In particular, hypertrophic cells may convert to apoptotic cells. For example, angiotensin II (Ang II) and tumor necrosis factor-α (TNF-α) can induce both hypertrophy and apoptosis (8, 9). Furthermore, apoptosis may play a driving role in the transition from compensated hypertrophy to failure in the work-overloaded myocardium (10). Although ARC has an anti-apoptotic function, it remains enigmatic whether it can influence cardiac hypertrophy.

Hypertrophy is conveyed by a complex signal transduction pathway. Reactive oxygen species (ROS) can mediate the hypertrophic signals. For example, TNF-α causes hypertrophy via the generation of ROS in cardiomyocytes (11). ROS can mediate the hypertrophic signals of phenylephrine (PE) (12) and Ang II (9). Although it is well known that catalase is a ROS scavenger, it remains largely unknown as to how catalase is integrated into the hypertrophic cascades.

Protein kinase CK2 (CK2) is a serine/threonine protein kinase ubiquitously expressed in eukaryotic cells. It plays a key role in cell cycle control, cellular differentiation, and proliferation. CK2 is characterized by its constitutive activation. It has been shown that CK2 phosphorylates ARC at Thr-149 (13, 14).
It is not yet clear whether CK2 can regulate cardiac hypertrophy.

Our present study showed that the expression levels of catalase were reduced in response to the hypertrophic stimulation. CK2 was oxidatively modified. As a consequence, ARC could not be phosphorylated and, thus, failed to inhibit the activation of mitochondrial permeability transition (MPT). Finally, hypertrophy was initiated. Our study reveals a novel anti-hypertrophic pathway that is composed of catalase, CK2, and ARC.

EXPERIMENTAL PROCEDURES

Materials—PE, cyclosporin A, SP600125, TNF-α, 4,5,6,7-tetramromo-2-azabenzimidazole (TBB), anti-dinitrophenyl (anti-DNP) antibody, catalase, N-acetyl-l-cysteine, pyrrolidine dithiocarbamate, and phalloidin-TRITC were purchased from Molecular Probes Inc. Anti-CK2-α antibody was from Upstate. Fostriecin and SB239063 were from Calbiochem. Anti-ARC antibody was from Chemicon. Anti-phospho Thr-149 antibody was from Eurogentec. CK2 was from Promega. The adenovirus harboring catalase was a kind gift from Dr. Joseph J. Cullen.

Preparations and Transfection of ARC Antisense Oligonucleotides—ARC antisense oligonucleotides were synthesized to inhibit endogenous ARC expression. The sequences of phosphothioate-modified antisense oligonucleotides targeted to ARC were ARC antisense oligonucleotides (ARC-AS), 5’-TGGGATGAGGGATGATGCT-3’; scrambled ARC antisense oligonucleotides (S-ARC-AS), 5’-GTAGGGTACGCCGATATGCT-3’; scrambled ARC antisense oligonucleotides (S-ARC-AS), 5’-GTAGGGTACGCCGATATGCT-3’; scrambled ARC antisense oligonucleotides (ARC-S), 5’-AGCTATGACCTCATGCCATA-3’. The specificity of the oligonucleotides was confirmed by comparison with all other sequences in GenBank™ using nucleotide BLAST. There was no homology to other known rat DNA sequences. Cells were transfected with the oligonucleotides using Lipofectin (Life Technology).

Constructions of Adenoviruses Harboring ARC, Catalase RNAi, or CK2α RNAi—The adenoviruses harboring the wild-type rat ARC (AdARC), an ARC mutant with Thr-149 converted to the alanine residue (AdT149A) and an ARC mutant with Thr-149 converted to an aspartic acid residue (AdARC149D) and an ARC mutant with Thr-149 converted to the alanine residue (AdT149A) were constructed using the Adeno-X™ expression system (Clontech). Viruses were amplified in HEK293 cells.

Cell Surface Area Measurement—Cell surface area of F-actin-stained cells or unstained cells was measured 48 h after employing hypertrophic stimuli by computer-assisted planimetry. 100–200 cardiomyocytes in 20–50 fields were examined in each experiment.

Cardiomyocytes Isolation and Culture—Cardiomyocytes were isolated from 1- to 2-day-old Wistar rats as described (16). Briefly, after dissection hearts were washed and minced in HEPES-buffered saline solution (containing (mM): 130 NaCl, 3 KCl, 1 NaH2PO4, 4 glucose, and 20 HEPES; pH adjusted to 7.35 with NaOH). Tissues were then dispersed in a series of incubations at 37°C in HEPES-buffered saline solution containing 1.2 mg/ml pancreatin and 0.14 mg/ml collagenase ( Worthington). After centrifugation cells were re-suspended in Dulbecco’s modified Eagle’s medium/F-12 (Amersham Biosciences) containing 5% heat-inactivated horse serum, 0.1 mM ascorbate, insulin-transferring-sodium selenite media supplement, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.1 mM bromoecdoryouridine. The dissociated cells were pre-plated at 37°C for 1 h. The cells were then diluted to 1 x 10^6 cells/ml and plated in 10 μg/ml laminin-coated different culture dishes according to the specific experimental requirements.

Angiotensinogen Transgenic Mice—Angiotensinogen transgenic mice were described elsewhere (17, 18). They were maintained as a heterozygous transgenic line on the NMRI genetic background (Charles River, Sulzfeld, Germany). Animals were maintained under standardized conditions with an artificial 12 h dark-light cycle, with free access to food and water. 9- to 10-week-old male and age-matched wild-type male mice were used in the experiments. This research was performed in compliance with the Guidelines for the Care and Use of Laboratory Animals of the local authorities.

CK2 Activity Assay—CK2 activity was measured as previously described (13). Reactions were carried out in buffer containing 50 mM Tris (pH 7.6), 100 mM NaCl, 20 mM MgCl2, 10 mM KCl, 5 μCi GTP, and 0.1 μM CK2 peptide (Arg-Arg-Glu-Glu-Thr-Glu-Glu-Glu, from Biomol).

Adenovirus Infection and CK2 Inhibition—Cardiomyocytes were infected with adenoviruses as previously described (15). In experiments employing CK2 inhibitor, cells were pretreated with TBB for 50 min prior to inducing hypertrophy.

Cell Surface Area Measurement—Cell surface area of F-actin-stained cells or unstained cells was measured 48 h after employing hypertrophic stimuli by computer-assisted planimetry. 100–200 cardiomyocytes in 20–50 fields were examined in each experiment.

[3H]Leucine Incorporation—Cardiomyocytes were infected with AdARC or Adβ-galactosidase. 24 h after infection they were treated with the hypertrophic stimuli for 48 h in the presence of [3H]leucine (1.0 μCi/ml). Thereafter, cells were washed three times with phosphate-buffered saline, incubated with 5% trichloroacetic acid for 20 min at 4°C, and lysed with 0.5 M NaOH. Scintillation fluid was applied to the lysates, and the mixtures were counted in a liquid scintillation counter.

Analysis of ANF Expression by Northern Blotting—Atrial natriuretic factor (ANF) expression was detected by Northern blotting as reported previously (19). Briefly, pre-hybridization was conducted at 42°C for 4 h in a pre-hybridization buffer: 50% formamide, 5 × SSC, 2% blocking reagent, 50 μM sodium phosphate, pH 7.4, 7% SDS (w/v), and 0.1% N-laurylsarkosine (w/v). Hybridization was performed in the same buffer and temperature for 30 h with digoxigenin-labeled ANF cDNA probe. For chemiluminescence detection, the membrane was
Catalase, CK2, and ARC in Regulating Hypertrophy

blocked for 30 min in 2.5% blocking reagent and then incubated for 30 min with anti-digoxigenin antibody conjugated with alkaline phosphatase. After two washes with 100 mM maleic acid buffer containing 0.3% Tween 20, CSPD substrate solution was added to the membrane, and the mixture was incubated for 10 min. The same membrane was stripped and re-probed with glyceraldehyde-3-phosphate dehydrogenase as a loading control.

Intracellular ROS and Mitochondrial Membrane Potential (Δψₘ) Analysis—Intracellular ROS levels were analyzed by employing ROS-sensitive dye, 2',7'-dichlorofluorescin diacetate as described (15). Dichlorofluorescein fluorescence was measured by flow cytometry. For the detection of Δψₘ, 100 nM tetramethylrhodamine ethyl ester was added, and the mixture was incubated for 15 min at 37°C. The fluorescence was measured by flow cytometry as described (20).

Immunoblotting—Immunoblotting was performed as described previously (13). In brief, cells were lysed for 1 h at 4°C in a lysis buffer (mM), 20 Tris (pH 7.5), 2 EDTA, 3 EGTA, 2 dithiothreitol, 250 sucrose, 0.1 phenylmethylsulfonyl fluoride, 1% Triton X-100, and a protease inhibitor mixture). The cultured cardiomyocytes were lysed for 1 h at 4°C in the lysis buffer. To perform immunoprecipitation, the lysates were precleared with 10% (v/v) protein A-agarose for 1 h at 4°C in the lysis buffer. To perform immunoprecipitation, samples were subjected to 12% SDS-PAGE and transferred to nitrocellulose membranes. Equal protein loading was controlled by Ponceau red staining of membranes. Blots were probed using antibodies.

Determination of CK2 Oxidative Modification—The mice hearts were homogenized in a lysis buffer (mM), 20 Tris (pH 7.5), 2 EDTA, 3 EGTA, 2 dithiothreitol, 250 sucrose, 0.1 phenylmethylsulfonyl fluoride, 1% Triton X-100, and a protease inhibitor mixture). The cultured cardiomyocytes were lysed for 1 h at 4°C in the lysis buffer. To perform immunoprecipitation, the lysates were pre-cleared with 10% (v/v) protein A-agarose for 1 h on a rocking platform. Anti-CK2-α antibody was added, and the mixture was rocked for 1 h. Immunoprecipitates were captured with 10% (v/v) protein A-agarose for another hour. For the detection of CK2-α protein using immunoblotting, samples were subjected to 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with the anti-CK2-α antibody for 1 h, and then with the horseradish peroxidase-conjugated secondary antibody. After detection of CK2-α protein, the membrane was stripped for the detection of CK2 carbonylation using the method described elsewhere (21). In brief, the membrane was equilibrated in 20% (v/v) methanol, 80% Tris-buffered saline for 5 min, and then incubated with 0.5 mM 2,4-dinitrophenylhydrazine for 30 min at room temperature. After washing, the membrane was incubated with the anti-DNP antibody. Antigen-antibody complexes were visualized by enhanced chemiluminescence.

TRITC-conjugated Phalloidin Staining—Actin filaments were visualized in cultured myocytes using TRITC-conjugated Phalloidin according to the manufacturer’s instructions, and imaged using a laser scanning confocal microscope (Zeiss LSM 510 META).

Statistical Analysis—The results are expressed as means ± S.E. The statistical comparison among different groups was performed by one-way analysis of variance. Paired data were evaluated by Student’s t test. p < 0.05 was considered statistically significant.

RESULTS

ARC Phosphorylation Levels, CK2 Activity, and Catalase Expression Levels Are Reduced in Cardiac Hypertrophy—Angiotensinogen transgenic mice have been shown to develop cardiac hypertrophy (18). We examined the expression and phosphorylation levels of ARC in these mice. As shown in Fig. 1 (A and B), ARC expression levels remained unchanged, whereas its phosphorylation levels were decreased in angiotensinogen transgenic mice. We detected the CK2 activity in these mice,

![Image](https://example.com/figure1.png)

FIGURE 1. ARC phosphorylation levels, CK2 activity, and catalase expression levels are reduced in hypertrophy. A, detection of ARC phosphorylation levels in angiotensinogen transgenic mice (TG). The heart lysates were used for immunoblot with anti-phospho Thr-149 antibody. The same membrane was stripped and re-probed with anti-ARC antibody. B, quantification analysis of ARC phosphorylation levels in angiotensinogen TG. The heart lysates from control mice (n = 6) and angiotensinogen TG (n = 11) were used to perform immunoblot using anti-phospho Thr-149 antibody and anti-ARC antibody. The results were densitometrically scanned using NIH ImageJ. *, p < 0.05 versus control. C, analysis of CK2 activity in control mice (n = 7) and angiotensinogen TG (n = 9). CK2 activity is expressed as mean ± S.E. of three independent experiments. H, analysis of catalase expression levels by immunoblot. The results are expressed as means ± S.E. of three independent experiments is shown.
and a reduction in CK2 activity was observed (Fig. 1C). The levels of catalase expression in these mice also were reduced (Fig. 1, D and E).

We further analyzed the levels of ARC expression and phosphorylation in cardiomyocytes upon treatment with the hypertrophic stimuli including PE, TNF-α, and Ang II. As shown in Fig. 1F, ARC protein levels were not altered, whereas its phosphorylation levels were reduced. Subsequently, we detected the CK2 activity, and observed a low level of CK2 activity in cardiomyocytes treated with PE, TNF-α, or Ang II (Fig. 1G). The expression levels of catalase were simultaneously reduced (Fig. 1H). Taken together, these data indicate that the levels of ARC phosphorylation, the activity of CK2, and the expression levels of catalase are decreased in the hypertrophic cascades.

**ARC Is Able to Inhibit Cardiomyocyte Hypertrophy**—The reduction in ARC phosphorylation levels in the hypertrophic cascades led us to consider whether there is a relationship between ARC and hypertrophy. To this end, we detected whether ARC could influence cardiomyocyte hypertrophy. The hypertrophic models were set up by employing PE, TNF-α, or Ang II as described elsewhere (9, 11). Hypertrophy is characterized by an enlargement of individual cardiomyocyte, an increase in protein synthesis, and the expression of fetal genes such as ANF. Cardiomyocytes treated with PE (Fig. 2A) or TNF-α (Fig. 2B) showed an increase in cell surface area, which could be attenuated by ARC. ARC also could attenuate the increase in [3H]leucine incorporation (Fig. 2, C and D). 25 nM Ang II-induced hypertrophy could be attenuated by ARC as assessed by cell surface area measurement and [3H]leucine incorporation (data not shown). ANF expression in response to the stimulation with PE, TNF-α, or Ang II could be inhibited by ARC (Fig. 2E). Thus, it appears that ARC is able to antagonize cardiomyocyte hypertrophy.

**Inhibition of Endogenous ARC Sensitizes Cells to Undergoing Hypertrophy**—To understand whether endogenous ARC participates in regulating hypertrophy, we detected the susceptibility of cells to undergoing hypertrophy upon ARC knockdown by its antisense oligonucleotides (ARC-AS). Scrambled ARC-AS oligonucleotides and ARC sense oligonucleotides (ARC-S) were used as controls. ARC-AS was able to induce a reduction in ARC levels (Fig. 3A) and observed a reduction in ARC phosphorylation for its anti-hypertrophic function. To this end, we first tested whether ARC requires Thr-149 phosphorylation (Fig. 4A). As shown in Fig. 4, phosphorylatable ARC149A on cardiomyocyte hypertrophy. Sarcomere organization is a dominant marker of hypertrophy in the cultured cardiomyocytes. As shown in Fig. 4A, PE could lead to sarcomere organization that was blocked by wtARC but not ARC149A. Next, we tested whether ARC149D could functionally mimic phosphorylation. ARC149D but not ARC149A was able to inhibit hypertrophy (Fig. 4B).
shown in Fig. 1 (C and G). PE, TNF-α, or Ang II are able to induce ROS production in cardiomyocytes (9). ROS can oxidatively modify proteins resulting in the loss of their functions (23). This led us to test whether CK2 could be oxidatively modified by ROS. Carbonylation is a marker for the oxidative modification of proteins (24). We analyzed the carbonylation of CK2 in the hearts of angiotensinogen transgenic mice and in cardiomyocytes treated with TNF-α. As shown in Fig. 5A, carbonylation of CK2 could be observed in the hearts of angiotensinogen transgenic mice. CK2 carbonylation could be detected in cells treated with TNF-α. The antioxidants, N-acetyl-L-cysteine (NAC) or pyrrolidine dithiocarbamate (PDTC), could attenuate CK2 carbonylation (Fig. 5B). Thus, CK2 is oxidatively modified in hypertrophy.

We asked whether the oxidative modification can lead to the loss of CK2 catalytic activity. To address this question, we firstly detected the catalytic activity of CK2 in cells treated with TNF-α in the absence or presence of antioxidants. As shown in Fig. 5C, the administration of NAC and PDTC could block the decrease in CK2 activity. Concomitantly, NAC and PDTC could inhibit TNF-α-induced hypertrophic responses as analyzed by cell surface area measurement (data not shown). We next analyzed whether the oxidative modification could directly alter CK2 activity. Hydrogen peroxide in the absence but not presence of catalase led to a reduction in CK2 activity (Fig. 5D). Taken together, these data suggest that the oxidative modification may be responsible for the loss of CK2 activity in the hypertrophic pathway.

Knockdown of Catalase Leads to CK2 Oxidative Modification and a Reduction in ARC Phosphorylation Levels—The simultaneous occurrences of the decreases in catalase expression, CK2 activity, and ARC phosphorylation levels in hypertrophy as
FIGURE 5. **CK2 is oxidatively modified.** A, analysis of CK2 carbonylation in the hearts of angiotensinogen TG. The heart lysates were used to perform immunoprecipitation. Immunoblot was performed to detect CK2-α using anti-CK2-α antibody (lower panel) and CK2 carbonylation using anti-DNP antibody (upper panel). B, analysis of CK2 carbonylation in cardiomyocytes treated with TNF-α in the absence or presence of antioxidants. Neuronal rat cardiomyocytes were pretreated with 0.5 mM NAC or 0.1 μM PDTC for 1 h and then treated with 10 ng/ml TNF-α for 1 h in the absence or presence of NAC or PDTC. Immunoblot was performed using anti-CK2-α antibody (lower panel), or anti-DNP antibody (upper panel). A representative result of three independent experiments is shown. C, antioxidants attenuate the decrease in CK2 activity upon treatment with TNF-α. The treatment of cardiomyocytes was as described in Fig. 5B. CK2 activity is expressed as cpm/mg of protein. *, p < 0.05 versus TNF-α alone. D, oxidative modification of CK2 in vitro. CK2 (30 units) was incubated for 1 h at 25 °C with 1 mM hydrogen peroxide or 25 mM Tris-HCl (pH 7.4) in the absence or presence of 100 units catalase. *, p < 0.05 versus CK2 alone. Data in C and D are expressed as the mean ± S.E. of three independent experiments.

shown in Fig. 1 led us to consider whether these events are causally linked with each other. We detected the status of CK2 carbonylation and ARC phosphorylation upon catalase knockdown. We used a low dose of catalase RNAi to avoid the occurrence of apoptosis. Treatment of cells with catalase RNAi but not its scrambled form (catalase-S-RNAi) resulted in the carbonylation of CK2 (Fig. 6A) and a reduction in ARC phosphorylation levels (Fig. 6B). Concomitantly, hypertrophy could be observed upon catalase knockdown (Fig. 6C). Thus, the decrease in catalase expression can lead to CK2-oxidative modification and a reduction in ARC phosphorylation levels.

**Catalase Can Maintain ARC Phosphorylation Levels and Attenuate Hypertrophy**—We subsequently tested whether catalase could influence ARC phosphorylation status in response to hypertrophic stimuli. Enforced expression of catalase could attenuate the reduction in ARC phosphorylation levels upon treatment with PE, TNF-α, or Ang II (Fig. 7A). Concomitantly, the increase in ROS levels (Fig. 7B) and hypertrophy (Fig. 7C) were attenuated by enforced expression of catalase. c-Jun N-terminal kinase (JNK) and p38 are involved in the hypertrophic cascades. We tested whether they could regulate ARC phosphorylation at Thr-149 and CK2 activity. SB239063 at 10 μM can effectively inhibit p38 (25). Inhibition of p38 with SB239063 at 10 μM led to no significant alterations in ARC phosphorylation levels and CK2 activity upon stimulation with PE, TNF-α, or Ang II (data not shown). SP600125 at 10 μM is able to significantly inhibit JNK (26). However, it cannot influence ARC phosphorylation levels and CK2 activity upon stimulation with PE, TNF-α, or Ang II (data not shown). To understand whether catalase influences ARC phosphorylation levels through a pathway by targeting protein phosphatase-2A or calcineurin, we detected ARC phosphorylation level in the presence of protein phosphatase-2A inhibitor Fostriecin or calcineurin inhibitor FK506. Inhibition of protein phosphatase-2A or calcineurin could not influence the effect of catalase on ARC phosphorylation. The cells were infected with AdCAcatalase at an m.o.i. of 50. 24 h after infection they were treated for 2 h with 1 μM Fostriecin or 1 μM FK506. Cells were then treated with 10 ng/ml TNF-α for 1 h. Immunoblot was performed with anti-phospho Thr-149 antibody and anti-ARC antibody. Data in B and C are expressed as the mean ± S.E. of three independent experiments.

**Catalase, CK2, and ARC in Regulating Hypertrophy**

![Catalase, CK2, and ARC in Regulating Hypertrophy](image-url)
whether ARC exerts its effect against cardiomyocyte hypertrophy by regulating MPT. We first detected the localization of ARC upon treatment with the hypertrophic stimuli. A decrease in the phosphorylated ARC levels in mitochondria-enriched heavy membrane was observed. An increase in nonphosphorylated ARC levels could be detected in the cytosol (Fig. 8A). Hence, ARC is redistributed in the hypertrophic pathway.

We detected the effect of ARC on MPT activation. Cyclosporin A was used as a positive control. $\Delta \psi_m$ was decreased in cardiomyocytes treated with TNF-$\alpha$. WtARC but not ARC149A was able to attenuate the decrease in $\Delta \psi_m$ (Fig. 8B).

To understand whether endogenous ARC can regulate MPT activation, we detected whether inhibition of endogenous ARC expression could influence MPT activation. As shown in Fig. 8C, a low dose of TNF-$\alpha$ (2 ng/ml) could not induce a significant alteration of $\Delta \psi_m$. In contrast, inhibition of endogenous ARC expression led to a decrease in $\Delta \psi_m$ upon treatment with 2 ng/ml TNF-$\alpha$. A similar result was observed in cells treated with a low dose of PE when ARC was inhibited (Fig. 8D). However, Ang II at a dose of 5 nm could induce hypertrophy but without affecting $\Delta \psi_m$ when ARC was inhibited (data not shown), suggesting that Ang II may induce hypertrophy through a distinct pathway.

To test whether phosphorylation is required for endogenous ARC to regulate $\Delta \psi_m$, we detected $\Delta \psi_m$ when endogenous ARC phosphorylation was inhibited by CK2 RNAi. 2 ng/ml TNF-$\alpha$ could lead to a decrease in $\Delta \psi_m$ in the presence of CK2 RNAi but not its scrambled form (CK2-S-RNAi) (Fig. 8E). A similar result was obtained in the hypertrophic model of PE at a low dose (Fig. 8F). Finally, we tested whether cyclosporin A could influence hypertrophy. TNF-$\alpha$-induced hypertrophy could be attenuated by cyclosporin A (Fig. 8G). Taken together, it appears that ARC may target MPT in the hypertrophic pathway.

**DISCUSSION**

Our present study reveals that the hypertrophic stimuli can down-regulate catalase resulting in an elevated level of ROS. The latter then oxidatively modifies CK2 leading to the loss of its catalytic activity. As a consequence, ARC loses its anti-hypertrophic function due to the reductive decrease of Thr-149 phosphorylation by CK2. Hence, our results reveal a novel anti-hypertrophic pathway that is composed of catalase, CK2, and ARC.

Although ROS plays an important role in the regulation of cardiac hypertrophy, the molecular mechanism by which ROS levels are elevated in response to the hypertrophic stimuli remains to be fully elucidated. The elevation of ROS levels can result from an increase in their production and/or a decrease in their decomposition. It has been well documented that the increase in their production can account for the elevation of ROS levels. For example, NADPH oxidase can produce ROS in response to hypertrophic stimuli (28). However, it remains largely unknown as to whether the decrease of ROS decomposition can contribute to the elevation of ROS levels in the hyper-
trophic pathway. Most recently, a report shows that sustained β-adrenergic receptor stimulation results in hypertrophy through transcriptionally down-regulating copper-zinc superoxide dismutase (29), a scavenger of superoxide. Our present study reveals that catalase, a scavenger of hydrogen peroxide, is down-regulated in response to the hypertrophic stimulation. Thus, it appears that the reduction of ROS decomposition also may contribute to the elevation of ROS levels in the hypertrophic cascades.

How can ROS mediate the hypertrophic signals? As second messengers, ROS can mediate the hypertrophic signals by regulating various intracellular signal transduction cascades and the activity of various transcription factors. For example, the activation of NF-κB and activator protein-1 can be controlled by ROS (9). ROS can mediate hypertrophic signals by activating mitogen-activated protein kinases (30–32) and apoptosis signal-regulating kinase 1 (28, 33). Our present study reveals that ROS can induce the oxidative modification of CK2 leading to the loss of its catalytic activity.

Our data show that ARC can inhibit hypertrophy. In particular, ARC requires phosphorylation at Thr-149 to exert its anti-hypertrophic effect. CK2 can phosphorylate ARC at Thr-149 (13, 14). The loss of the CK2 activity results in the reduction in ARC phosphorylation. These data indicate that CK2 and ARC form an axis in regulating hypertrophy, and ROS may target this axis.

ARC is an anti-apoptotic protein. How can one molecule prevent two distinct cellular events such as hypertrophy and apoptosis? Apoptosis and hypertrophy can be initiated through the mitochondrial pathway. In hypertrophy, for example, the maintenance of mitochondrial integrity can protect cells from undergoing hypertrophy. Mitochondrial K_{ATP} Channel activation could represent an effective approach to minimize the myocardial hypertrophic process (34–36). Desmin has been shown to play an important role in the maintenance of cellular integrity of muscle tissues (37). Desmin null mice (des^{-/-}) develop cardiac hypertrophy with mitochondrial abnormalities. Bcl-2 overexpression in the des^{-/-} heart corrects the mitochondrial defects and ameliorates cardiac hypertrophy (38). Furthermore, overexpression of the serotonin 5-HT2B receptor in heart leads to abnormal mitochondrial function and cardiac hypertrophy (39). Our present study demonstrates that ARC can maintain MPT. Thus, it appears that the maintenance of the mitochondrial integrity by ARC couples its anti-hypertrophic and anti-apoptotic functions.

It is noteworthy that overexpression of some anti-apoptotic factors can lead to the development of cardiac hypertrophy. For example, cytoplasmic overexpression of Akt in mice results in myocyte hypertrophy, whereas nuclear-targeted Akt does not lead to cardiac hypertrophy (40–42). In contrast, overexpression of other anti-apoptotic factors such as Bcl-2 can prevent hypertrophy (38). Our present work showed that ARC, as an anti-apoptotic protein, can inhibit cardiac hypertrophy. This is consistent with the results of Bcl-2. One explanation for such distinct effects of the anti-apoptotic proteins on hypertrophy is the different molecular consequences of their activation. For example, a variety of factors that control cardiomyocyte growth are substrates of Akt (43), whereas ARC and Bcl-2 have not yet been shown to be able to activate cardiomyocyte growth factors.

The previous work shows that the hearts from ARC-deficient mice underwent no obvious hypertrophy but apoptosis and dilatation in the model of transverse aortic banding (44). It is possible that the hearts in the absence of ARC are more susceptible to undergoing apoptosis without the transition from compensatory hypertrophy to decompensatory dilatation. It remains to be re-evaluated whether ARC-deficient mice are susceptible to undergoing cardiac hypertrophy under different conditions. The previous work showed that ARC is unable to inhibit hypertrophy induced by a high dose of Ang II (44). The molecular mechanism accounting for this disability of ARC to inhibit hypertrophy induced by the high dose of Ang II remains to be elucidated.

Our data showed that the expression levels of catalase were reduced upon stimulation with the hypertrophic stimuli. Furthermore, knockdown of catalase could lead to the oxidative modification of CK2. This indicates that the oxidative modification of CK2 can be a result of the decrease in ROS decomposition. It would be interesting to elucidate whether ROS from other sources such as NADPH oxidase also contribute to the oxidative modification of CK2.

The present study reveals that in cardiomyocytes there exists an anti-hypertrophic pathway composed of catalase, CK2, and ARC. These three molecules cooperate to antagonize cardiac hypertrophy. Our results could lead to further studies to explore the beneficial effect of this pathway as a biological target for the treatment of maladaptive hypertrophy as well as heart failure.

REFERENCES
1. Koseki, T., Inohara, N., Chen, S., and Nunez, G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5156–5160
2. Nam, Y. J., Mani, K., Ashton, A. W., Peng, C. F., Krishnamurthy, B., Hayakawa, Y., Lee, P., Korsmeyer, S. J., and Kitsis, R. N. (2004) Mol. Cell 15, 901–912
3. Gustafsson, A. B., Tsai, J. G., Logue, S. E., Crow, M. T., and Gottlieb, R. A. (2004) J. Biol. Chem. 279, 21233–21238
4. Gustafsson, A. B., Sayen, M. R., Williams, S. D., Crow, M. T., and Gottlieb, R. A. (2002) Circulation 106, 735–739
5. Ekhterae, D., Lin, Z., Lundberg, M. S., Crow, M. T., Brosius, F. C. R., and Nunez, G. (1999) Circ. Res. 85, 670–677
6. Neuss, M., Monticone, R., Lundberg, M. S., Chesley, A. T., Fleck, E., and Crow, M. T. (2001) J. Biol. Chem. 276, 33915–33922
7. Geertman, R., McMahon, A., and Sabban, E. L. (1996) Biochim. Biophys. Acta 1306, 147–152
8. Barlucchi, L., Leri, A., Dostal, D. E., Fiordaliso, F., Tada, H., Hintze, T. H., Kajstura, J., Nadal-Ginard, B., and Anversa, P. (2001) Circ. Res. 88, 298–304
9. Hirotani, S., Otsu, K., Nishida, K., Higuchi, Y., Morita, T., Nakayama, H., Yamaguchi, O., Mano, T., Matsumura, Y., Ueno, H., Tada, M., and Hori, M. (2002) Circulation 105, 509–515
10. Crow, M. T., Mani, K., Nam, Y. J., and Kitsis, R. N. (2004) Circ. Res. 95, 957–970
11. Nakamura, K., Fushima, K., Kouchi, H., Mihara, K., Miyazaki, M., Ohe, T., and Namba, M. (1998) Circulation 98, 794–799
12. Tanaka, K., Honda, M., and Takabatake, T. (2001) J. Am. Coll. Cardiol. 37, 676–685
13. Li, P. F., Li, J., Muller, E. C., Otto, A., Dietz, R., and von Harsdorff, R. (2002) Mol. Cell 10, 247–258
14. Zhang, Y. Q., and Herman, B. (2006) J. Cell. Biochem. 99, 575–588
15. Li, P. F., Dietz, R., and von Harsdorff, R. (1999) EMBO J. 18, 6027–6036

Catalase, CK2, and ARC in Regulating Hypertrophy
Catalase, CK2, and ARC in Regulating Hypertrophy

16. von Harsdorf, R., Li, P. F., and Dietz, R. (1999) Circulation 99, 2934–2941
17. Kimura, S., Mullins, J. I., Bunnemann, B., Metzger, R., Hilgenfeldt, U., Zimmermann, F., Jacob, H., Fuxe, K., Ganten, D., and Kaling, M. (1992) EMBO J. 11, 821–827
18. Kang, N., Walther, T., Tian, X. L., Bohlender, J., Fukamizu, A., Ganten, D., and Bader, M. (2002) J. Mol. Med. 80, 359–366
19. Li, P. F., Dietz, R., and von Harsdorf, R. (1997) Circulation 96, 3602–3609
20. Li, J., Li, P. F., Dietz, R., and von Harsdorf, R. (2002) Apoptosis 6, 511–517
21. Conrad, C. C., Talent, J. M., Malakowsky, C. A., and Gracy, R. W. (2000) Biol. Procedures Online 2, 39–45
22. Loizou, J. I., El-Khamisy, S. F., Zlatanou, A., Moore, D. J., Chan, D. W., Qin, J., Sarno, S., Meggio, F., Pinna, L. A., and Caldecott, K. W. (2004) Cell 117, 17–28
23. Stadtman, E. R. (2001) Ann. N. Y. Acad. Sci. 928, 22–38
24. Grune, T., Merker, K., Jung, T., Sitte, N., and Davies, K. J. (2005) Free Radic. Biol. Med. 39, 1208–1215
25. Morissette, M. R., Cook, S. A., Foo, S., McKoy, G., Ashida, N., Novikov, M., Scherrer-Crosbie, M., Li, L., Matsui, T., Brooks, G., and Rosenzweig, A. (2006) Circ. Res. 99, 15–24
26. Guo, J., Sabri, A., Elouardighi, H., Rybin, V., and Steinberg, S. F. (2006) Circ. Res. 99, 1367–1375
27. Javadov, S., Huang, C., Kirshenbaum, L., and Karmazyn, M. (2005) J. Mol. Cell. Cardiol. 38, 135–143
28. Zhou, C., Ziegler, C., Birdie, L. A., Stewart, A. F., and Levitan, E. S. (2006) Circ. Res. 98, 1040–1047
29. Srivastava, S., Chandrasekar, B., Gu, Y., Luo, J., Hamid, T., Hill, B. G., and Prabhu, S. D. (2007) Cardiovasc. Res. 74, 445–455
30. Molkentin, J. D. (2004) Cardiovasc. Res. 63, 467–475
31. Kuster, G. M., Pimentel, D. R., Adachi, T., Ido, Y., Brenner, D. A., Cohen, R. A., Liao, R., Siwik, D. A., and Colucci, W. S. (2005) Circulation 111, 1192–1198
32. Nishida, M., Maruyama, Y., Tanaka, R., Kontani, K., Nagao, T., and Kurose, H. (2000) Nature 408, 492–495
33. Izumiya, Y., Kim, S., Izumi, Y., Yoshida, K., Yoshiyama, M., Matsuzawa, A., Ichijo, H., and Iwao, H. (2003) Circ. Res. 93, 874–883
34. Xia, Y., Rajapurohitam, V., Cook, M. A., and Karmazyn, M. (2004) J. Mol. Cell. Cardiol. 37, 1063–1067
35. O’Rourke, B. (2004) Circ. Res. 94, 420–432
36. Weiss, J. N., Korge, P., Honda, H. M., and Ping, P. (2003) Circ. Res. 93, 292–301
37. Capetanaki, Y., and Milner, D. J. (1998) Subcell. Biochem. 31, 463–495
38. Weisleder, N., Taffet, G. E., and Capetanaki, Y. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 769–774
39. Nebigil, C. G., Jaffre, F., Messaddeq, N., Hickel, P., Monassier, L., Launay, J. M., and Maroteaux, L. (2003) Circulation 107, 3223–3229
40. Rota, M., Boni, A., Urbanek, K., Padin-iruegas, M. E., Kajstura, T. J., Fiore, G., Kubo, H., Sonnenblick, E. H., Musso, E., Houser, S. R., Leri, A., Sussman, M. A., and Anversa, P. (2005) Circ. Res. 97, 1332–1341
41. Condorelli, G., Drusco, A., Stassi, G., Bellacosa, A., Roncarati, R., Iaccarino, G., Russo, M. A., Gu, Y., Dalton, N., Chung, C., Latronico, M. V. G., Napoli, C., Sadoshima, J., Croce, C. M., and Ross, J., Jr. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12333–12338
42. Shi, T., McMullen, J. R., Kang, P. M., Douglas, P. S., Obata, T., Franke, T. F., Cantley, L. C., and Iruquo, S. (2002) Mol. Cell. Biol. 22, 2799–2809
43. Heineke, J., and Molkentin, J. D. (2006) Nat. Rev. Mol. Cell. Biol. 7, 589–600
44. Donath, S., Li, P. F., Willenbockel, C., Al-Saadi, N., Gross, V., Willnow, T., Bader, M., Martin, U., Bauersachs, J., Wellens, K. C., Dietz, D., and von Harsdorf, R. (2006) Circulation 113, 1203–1212