Mitochondrial Dysfunction and Apoptosis Underlie the Pathogenic Process in α-B-Crystallin Desmin-Related Cardiomyopathy

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Background—Mitochondria and sarcomeres have a well-defined architectural relation that partially depends on the integrity of the cytoskeletal network. An R120G missense mutation in the small heat shock protein α-B-crystallin (CryAB) causes desmin-related cardiomyopathy. Desmin-related cardiomyopathy is characterized by the formation of intracellular aggregates containing CryAB and desmin that are amyloid positive, and disease can be recapitulated in transgenic mice by cardiac-specific expression of the mutant protein.

Methods and Results—To understand the resultant pathology, we explored the acute effects of R120G expression both in vitro and in vivo. In vitro, transfection of adult cardiomyocytes with R120G-expressing adenovirus resulted in altered contractile mechanics. In vivo, as the cytoskeletal network is disturbed but before deficits in organ function can be detected, alterations in mitochondrial organization and architecture occur, leading to a reduction in the maximal rate of oxygen consumption with substrates that utilize complex I activity, alterations in the permeability transition pore, and compromised inner membrane potential. Apoptotic pathways are subsequently activated, which eventually results in cardiomyocyte death, dilation, and heart failure.

Conclusions—Cardiac chaperone dysfunction acutely leads to altered cardiomyocyte mechanics, perturbations in mitochondrial-sarcomere architecture, and deficits in mitochondrial function, which can result in activation of apoptosis and heart failure. (Circulation. 2005;112:3451-3461.)

Key Words: cardiomyopathy ■ heart diseases ■ heart failure ■ molecular biology

Mutations in desmin, α-B-crystallin (CryAB), and other genes result in the desmin-related myopathies (DRM).1,2 The desminopathies are characterized by accumulations of electron-dense granulofilamentous aggregates in the skeletal and cardiac myocytes. These amorphous bodies contain desmin, CryAB, and other proteins that may or may not be shared across the diverse pathologies. Patients normally have distal weakness in their limbs and often show cardiac hypertrophy, conduction block, and arrhythmias.3 Although the initial focus centered on mutations in the intermediate filament protein desmin, it has become apparent that mutations in other proteins that interact with desmin, such as the R120G mutation in CryAB (CryABR120G), can phenocopy the disease.4 We recently modeled CryABR120G cardiomyopathy in the mouse by cardiac-specific transgenic expression and showed that CryABR120G expression causes heart failure by 5 to 7 months.5

CryAB is a member of the small heat shock protein family, and, although it was originally classified as a lens protein, CryAB is present at high concentrations in both cardiac and skeletal muscle and in lower concentrations in brain, skin, and kidney. CryAB has chaperone-like, anti-aggregation properties, binds to both desmin and cytoplasmic actin, and helps to maintain cytoskeletal integrity. As is the case for other chaperones and the small heat shock proteins, CryAB can bind to unfolded proteins and prevent their denaturation and aggregation. During ischemia, CryAB also binds to the contractile apparatus, presumably protecting the components from denaturation.6

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Although the genetic pathologies for the DRMs have now been partially defined, the pathogenic sequelae remain obscure, particularly for the CryABR120G-based pathology. The mouse model revealed a collapse of the desmin network, and this undoubtedly contributes to the developing disease, yet
similar structural changes in the cardiomyocyte cytoskeleton in another DRM animal model, which expressed a desmin mutation, resulted in significantly less morbidity and no mortality. Therefore it seems likely that expression of mutant CryAB has other effects, in addition to leading to alterations in the desmin network, and indeed we noted that aggregates in the CryABR120G cardiomyocytes invariably contain amyloid-like material, whereas those in the desmin mutant cells do not.

In comparing the 2 models, CryABR120G cardiomyocyte mitochondrial organization and architecture appeared to be disproportionately affected in the young adults (Villa and Robbins, unpublished data, 2005). Mitochondrial organization is rigidly controlled in both skeletal and cardiac myocytes and depends on the integrity of the cytoskeleton. To explore whether early pathological processes involved mitochondrial dysfunction and to determine the pathogenic basis for the heart failure observed in the CryABR120G mice, we have now defined the acute and chronic effects of cardiomyocyte CryABR120G expression in vitro and in vivo. CryABR120G expression quickly leads to visible aggregates, and their accumulation results in deficits in contractile behavior as the cell contracts at high rates. These acute effects are rapidly followed by alterations in mitochondrial-sarcomere architecture and function, which eventually lead to activation of apoptotic pathways that further compromise cardiomyocyte function and viability, resulting in the development of heart failure and death.

**Methods**

**Transgenic Mice**

FVB/N mice with cardiac-specific overexpression of normal CryAB (wild type) or CryAB containing the R120G missense mutation (CryABR120G), driven by the α-myosin heavy chain promoter, have been described. Transgenic mice were identified by polymerase chain reaction analysis of DNA isolated from tail clips.

**Antibodies**

Anti-Caspase-3 antibody and anti-VDAC were obtained from Abcam, Inc. Anti-cytochrome c was purchased from BD Biosciences and anti-cTnl antibody (MAB1691) from Chemicon.

An expanded Methods section is included as Online Supplemental Material.

**Results**

**CryABR120G Expression Affects Cardiomyocyte Contractility**

We wished to determine whether CryABR120G expression had acute effects on cardiomyocyte mechanics. Changes in cardiomyocyte contractility can be caused by altered sarcomeric function, modifications in passive cytoskeletal stiffness, or structural changes in sarcomere organization. To understand the acute effects of CryABR120G on contractility, isolated adult cardiomyocytes were transfected with adenoviruses carrying CryABR120G or wild-type (WT) CryAB. In preliminary experiments, we found that a multiplicity of infection of 100 yielded a level of overexpression that approximated that observed in the CryABR120G mice previously reported; when quantified, protein accumulations did not differ significantly between the CryABR120G and CryABWT cultures (Figure 1A). The cells tolerated significant levels of CryAB expression, with both the overall morphology and integrity of the contractile apparatus preserved (Figure 1B), consistent with previous data that confirmed cell integrity and the lack of nonspecific structural or functional changes on adenomederated gene transfer. Cells transfected with CryABR120G showed the characteristic punctate pattern of densely staining material consistent with early aggregate formation (Figure 1B). The cells were then paced at either 0.2 or 2 Hz and the magnitude and velocity of shortening and relaxation were determined. Strikingly, after only 4 days of CryABR120G expression, those cardiomyocytes showed marked arrhythmias on pacing at the faster rate (Figure 1C). Additionally, when paced at 2 Hz, both peak shortening and the maximum departure velocity were significantly less in the CryABR120G cells (Figure 1, C through E). Function in the CryABWT transfected cells was comparable to control myocytes and confirmed that the effects observed were specific to the mutant protein.

**Mitochondrial Alterations Result From CryABR120G Expression**

Intermediate filaments associate with mitochondria in many cell types, including skeletal and cardiac myocytes. In the last decade, using a number of different approaches including loss of function, it has become apparent that the cytoskeleton in general and the intermediate filaments in particular play an important role in mitochondrial localization and transport. In our previous analyses of cardiac tissue from CryABR120G mice, we noted that disruption of the desmin network occurred early in 3-month adults with detectable aggregation of desmin and CryAB. To assess whether mitochondrial ultrastructure was also affected by CryABR120G expression, we examined cardiomyocyte ultrastructure at 6 weeks, when the sarcomeric architecture is fully developed (Figure 2). At this time, no functional deficits as analyzed by echocardiography or histological abnormalities could be detected, nor had any detectable hypertrophy occurred. Although electron microscopy did not show any gross alterations in mitochondrial morphology, the overall architecture of the mitochondrial arrangement with the sarcomeres was significantly altered in the CryABR120G cardiomyocytes. The normal arrangement, in which the mitochondria are packed between the sarcomeres in a well-ordered array with their transverse boundaries tightly linked to the Z line, was perturbed, with bundles of unaligned mitochondria frequently apparent (Figure 2A). Although there were obvious morphological changes in the gross architecture of the mitochondria, most appeared to be relatively intact, with dense, well-ordered cristae, although mitochondria immediately surrounding nascent aggregates were clearly affected. To ascertain their functional state, mitochondria were isolated from the nontransgenic and transgenic hearts and the maximum rate of ADP stimulated oxygen consumption (Vmax) with glutamate and malate as substrate determined. Surprisingly, even at this early stage when cardiac disease is not apparent, Vmax was reduced by ≈50% in mitochondria derived from the
CryABR120G hearts (Figure 2B). To identify the site of the defect in respiration, we examined the maximal uncoupler stimulated respiration rate with substrates that enter at different sites along the electron transport chain. Glutamate and malate generate NADH, which enters through the NADH dehydrogenase-complex I site. With glutamate/malate as substrate, the transgenic mitochondria exhibited significantly lower rates of uncoupler stimulated oxygen consumption, consistent with the reduced V_{max} for oxygen consumption in the transgenic mitochondria. Interestingly, with either succinate or ascorbate + N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) as substrate, there was no difference in oxygen consumption between nontransgenic or transgenic mitochondria. Succinate enters the electron transport chain via succinate dehydrogenase or complex II. TMPD can donate electrons directly to cytochrome c, thereby bypassing complexes I, II, and

**Figure 1.** Contractile function of adult cardiomyocytes transfected with wild-type or mutant CryAB. A, Representative histogram of Western blots of CryABWT and CryABR120G expression in transfected cardiomyocytes as a function of the multiplicity of infection (MOI). MOIs of 100 resulted in protein expression equivalent to that observed in the transgenic mouse model of DRM. B, Immunofluorescent images of adult rat cardiomyocytes 5 days after infection. Conservation of the contractile apparatus is apparent. Control indicates cardiomyocytes that were transfected at the same MOI with vehicle only. C, Representative steady-state single recordings of sarcomere shortening obtained in control myocytes (left panel) and myocytes expressing CryABR120G (right panel) stimulated at 0.2 Hz (60 seconds, upper traces) and 2.0 Hz (15 seconds, lower traces). Note the inability of myocytes expressing CryABR120G to maintain pacing at 2.0 Hz. Recordings from myocytes expressing CryABWT were not significantly different from control recordings. D, Composite sarcomere shortening traces for myocytes from control, CryABWT, and CryABR120G groups paced at 2 Hz. These traces demonstrate the significant decrease in peak shortening, departure velocity, and +dP/dt\text{max} for myocytes expressing CryABR120G as shown in C. Composite traces for CryABR120G myocytes do not include stimuli that were not followed by cell shortening. Baseline sarcomere length, departure, and return velocity, +dP/dt\text{max}, and peak shortening were not significantly different in myocytes from either the control, CryABWT, or CryABR120G transfected cells at 0.2 Hz. E, Analysis of contractile shortening in myocytes from control (n=39), CryABWT (100 MOI, n=26), and CryABR120G (100 MOI, n=29) groups. Peak shortening, departure velocity, and +dP/dt\text{max} decreased significantly (P<0.05) in myocytes expressing CryABR120G compared with control and wild-type CryAB myocytes.
III. These data point to a specific defect in mitochondrial complex I in the CryABR120G hearts, as substrates that enter downstream of complex I show similar rates of oxygen consumption in the nontransgenic and transgenic mitochondria. To examine whether this defect in maximal oxygen respiration was an artifact due to isolation of the mitochondria, we also measured the maximum rate of respiration with glutamate and malate as substrate in

![Figure 2. CryABR120G expression leads to alterations in cardiac mitochondrial organization and function. A, Shown are electron micrographs derived from 6-week mice expressing equal levels of either CryABWT or CryABR120G. Note the abnormal architectural relation of the mitochondria to the sarcomeres in the CryABR120G cardiomyocytes. B, CryABR120G mitochondria have impaired respiration at complex I (NADH dehydrogenase complex). Vmax for mitochondrial oxygen consumption is significantly reduced in both isolated mitochondria and in situ mitochondria (saponin treated myocytes; myo) derived from CryABR120G hearts. n=3 to 4 for isolated mitochondria data and n=4 to 5 for saponin-treated myocytes. *P<0.05 for nontransgenic versus transgenic. C, Ca²⁺-induced swelling in isolated mitochondria. Mitochondria were isolated from the hearts at 2 months or 6 months, primed with 25 μmol/L CaCl₂ and swelling induced by the addition of 200 mmol/L CaCl₂ (n=3). D, ³¹P-NMR spectra were used to evaluate phosphocreatine levels in 8 week CryABWT and CryABR120G Langendorff-perfused hearts.](http://circ.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.105.566699?journalCode=cir)
in the hearts from 6-month-old CryABR120G transgenic mitochondria.

Despite the lack of obvious pathology at 6 to 8 weeks, we reasoned that if the mitochondria were truly involved in a primary pathogenic response, this should be manifested at the whole organ level. To that end, 31P-NMR spectra were used to evaluate phosphocreatine (Pcr) levels in 8-week CryABWT and CryABR120G Langendorff-perfused hearts (Figure 2D). Decreased Pcr/ATP ratios are a predictor of mortality in patients with cardiomyopathy,14 and we reasoned, on the basis of the above data, that this ratio would be affected in the mice. Even at this early age, we observed a significant decrease in Pcr/ATP in the CryABR120G hearts (1.60±0.08, n=8) compared with CryABWT hearts (1.96±0.09, n=8, P<0.05).

Considering the early appearance of mitochondrial dysfunction, we suspected that there might be a direct association of the normal or mutant protein with mitochondria. Cytosolic and mitochondrial fractions were isolated and the protein composition of each determined with respect to CryAB and desmin concentrations. Strikingly, the mitochondrial fraction derived from the CryABR120G hearts contained a significantly higher percentage of total CryAB compared with either the nontransgenic or CryABWT-transgenic material, whereas desmin levels were only modestly higher (Figure 3A). On the basis of the data presented in Figure 2, we suspected a specific interaction of CryABR120G with components of the PTP and consistent with this hypothesis, immunoprecipitation with VDAC shows a strong interaction with CryABR120G but almost none with CryAB derived from Ntg or CryABWT overexpressing hearts (Figure 3B). Western analysis for the presence of desmin in the anti-VDAC–precipitated material confirmed that nonspecific contamination with the desmin-positive aggregates, which also contain CryAB, was not responsible for the CryAB-positive signal (Figure 3B). We conclude that CryABR120G can strongly interact with VDAC in the mice before overt disease presents.

Activation of Apoptosis in CryABR120G Mice

Mitochondrial permeability transition and swelling can result in cytochrome c release and subsequent activation of apoptosis. We measured cytochrome c in both the cytosolic and mitochondrial fractions and observed progressive release of cytochrome c into the cytoplasm as pathology progressed (Figure 4A). Cytochrome c was present exclusively in the mitochondrial fractions of nontransgenic and 2- to 4-month-old CryABR120G mice. By 6 months, when the transgenic mice show acute symptoms of heart failure,5 cytochrome c was observed almost exclusively in the cytosolic compartment. To confirm the activation of apoptotic processes in the CryABR120G cardiomyocytes, we examined caspase-3 cleavage in the hearts from 5- to 6-month-old nontransgenic and CryABR120G mice. Confocal microscopy (Figure 4B) and Western analysis (Figure 4C) confirmed high levels of caspase-3 activation in the CryABR120G hearts during the latter stages of the disease and the absence of any detectable caspase-3 activity in the comparable nontransgenic hearts. TUNEL assays carried out on hearts derived from 5-month-
old CryAB<sup>R120G</sup> and CryAB<sup>WT</sup> mice confirmed the elevated apoptotic indices were due to overexpression of the mutant protein and not simply reflecting a generalized response to high levels of CryAB (Figure 4D and 4E).

### CryAB<sup>R120G</sup> Expression Affects the Mitochondrial Permeability Transition Pore

To confirm that the mitochondrial alterations and activation of cell death pathways could be acutely activated by CryAB<sup>R120G</sup> overexpression, rat neonatal cardiomyocytes (RNC) were transfected with adenoviruses containing CryAB-<sup>WT</sup> or CryAB<sup>R120G</sup>. Dissipation of mitochondrial membrane potential (Δψ) is a critical event in the process of cell death. To examine whether CryAB<sup>R120G</sup> overexpression was associated with changes in Δψ, we measured the ability of the transfected cells’ mitochondria to take up and retain the mitochondrion-selective dye tetra-methyl-rhodamine ester (TMRE). TMRE is a fluorescent probe whose accumulation and subsequent retention is dependent on Δψ and is used to image time-dependent mitochondrial membrane potential. Figure 5A shows representative histograms and density plots from RNC that were transfected with either CryAB<sup>WT</sup> or CryAB<sup>R120G</sup>-containing adenovirus and subjected to FACS analysis. Overexpression of CryAB<sup>R120G</sup> decreased TMRE fluorescence and shifted the distribution curve leftward, indicating depolarization of Δψ, relative to either the control RNC or those transfected with CryAB<sup>WT</sup>. The summarized data (Figure 5B) show that approximately 90% of nontransfected and 70% of CryAB<sup>WT</sup>-transfected RNC retained high levels of fluorescence as compared with 9% of the CryAB<sup>R120G</sup>-transfected cardiomyocytes (P<0.0001 versus control).

To characterize PTP activation at the single cardiomyocyte level, time-lapse confocal microscopy was performed with TMRE-loaded RNC. Nontransfected, CryAB<sup>WT</sup>- or CryAB<sup>R120G</sup>-transfected cardiomyocytes were treated with the mitochondrial uncoupler, CCCP, and time-dependent TMRE loss monitored by time-lapse scanning (Figure 6). Sequential images obtained from individual cells showed the rapid loss of signal from the CryAB<sup>R120G</sup>-transfected cells, relative to those transfected with CryAB<sup>WT</sup>. CryAB<sup>R120G</sup> expression not only decreased cardiomyocyte Δψ but also accelerated the onset of TMRE loss, suggesting that PTP opening had already occurred. CsA treatment of the CryAB<sup>R120G</sup> RNC confirmed that the rapid loss observed was due to PTP opening (Figure 6B). The calculated time to 50% percent of TMRE loss in CryAB<sup>R120G</sup>-transfected myocytes was 95 seconds compared with approximately 200 seconds in the nontransfected or CryAB<sup>WT</sup> transfected cardiomyocytes (Figure 6C).

Considering the effects on mitochondrial morphology, architecture and function, as well as the appearance of apoptotic cells in the CryAB<sup>R120G</sup> hearts, we examined whether CryAB<sup>R120G</sup> expression also led to activation of apoptosis in transfected RNC. Apoptotic cell death was clearly present in transfected cardiomyocytes as indicated by the appearance of early (annexin V), mid (caspase-3), and late (TUNEL) apoptotic markers (Figure 7A). Essentially all of the transfected cardiomyocytes stained positively for annexin

![Figure 4. Apoptotic pathways are activated in CryAB<sup>R120G</sup> mice. A, Partitioning of cytochrome c between the cytosolic and mitochondrial fractions. As the CryAB<sup>R120G</sup> mice age, cytochrome c is released from the mitochondria. The appearance of cytosolic cytochrome c was not due to contamination of the fraction with mitochondria, as the mitochondrial protein VDAC was found only in the mitochondrial fraction. B, Immunostaining for activated caspase-3 (appears as yellow) in sections derived from 5-month hearts. Cardiomyocytes were identified by phallolidin staining for actin (red); caspase-3 activity (arrows) is apparent in the CryAB<sup>R120G</sup> section (transgenic). C, Activated caspase-3 levels in ventricular homogenates from nontransgenic (2 and 6 months) and CryAB<sup>R120G</sup> (transgenic) mice (2, 4, 5, 6 months) were detected by Western analysis. D, TUNEL assays in 5-month hearts derived from CryAB<sup>WT</sup> and CryAB<sup>R120G</sup> mice. Cardiomyocytes were identified by phallolidin staining for actin (green). The positive signal was apparent only in the CryAB<sup>R120G</sup>-derived sections and is indicated by an arrow; this region is magnified ×3 and shown in the white bordered area. E, Quantification of TUNEL signal. Between 3 and 12×10<sup>5</sup> nuclei were counted for each sample and the percent TUNEL positive cells determined.](http://circ.ahajournals.org/content/circres/105/18/e29460/F4.large.jpg)
V and quantitation of caspase-3 showed a 3.5-fold upregulation in CryABR120G-transfected RNC as compared with non-transfected cardiomyocytes (Figure 7B).

**Discussion**

Desmin-related myopathy is a subgroup of myofibrillar myopathy caused by mutations in desmin, CryAB, and other proteins that interact with the intermediate filaments. The pathology is characterized by myofibril disruption that appears to initiate at the Z-disk. Dislocation and aggregation of membranous organelles is observed as well as the accumulation of the mutant, misfolded desmin, and/or CryAB into insoluble aggregates, which gradually increase in the cytoplasm and are thought to eventually result in cell death.1–3 We previously showed that these aggregates may be classified as aggresomes, whose accumulation is often associated with neurodegenerative diseases caused by protein misfolding or unfolding.8 Our studies showed that the aggresomes present in the cardiomyocytes contain
large concentrations of a toxic amyloid oligomer, which is typically found in many of the amyloid-based neurodegenerative diseases. The data thus link these cardiomyopathies to a broad class of amyloid-based neurodegenerative disease and offer potential insight into the mechanistic bases for the cardiac pathology that eventually results in dilation and death by heart failure.5

A loss of desmin or CryAB function has been thought to be an underlying cause for the development of cardiomyopathy and heart failure in DRM patients due to either the inability of the mutant desmin to maintain cytoskeletal integrity or by the loss by CryAB chaperone function, which would subsequently lead to desmin misfolding and eventual formation of the characteristic aggregates. Although loss of function may indeed contribute to the pathology, it cannot explain it completely, as we have noted a relatively benign cardiac phenotype, compared with the CryABR120G animals, in homozygous CryAB knockout mice (Maloyan and Robbins, unpublished observations, 2005). We think it likely that CryABR120G expression leads to a multifocal pathology. There appears to be physical and mechanical repercussions of CryABR120G expression, probably because of the development of the small protein aggregates. Viral transfection into adult rat myocytes shows that the acute effects of CryABR120G expression result in significant deficits in both peak shortening and maximum departure velocity with irregular contraction when pacing is increased to 2 Hz (Figure 1). In addition to the altered cardiomyocyte mechanics in Figure 1, we showed previously that in transfected cardiomyocytes amyloid oligomer was present within 48 to 60 hours after transfection8 and in the CryABR120G mice as soon as 2 to 3 days after the transgene is activated (Robbins, unpublished data, 2005). However, even in the intensely studied neurodegenerative disease processes thought to be due to amyloid oligomer toxicity, the exact sequence of events leading from amyloid formation to cell death is unknown. Several potentially damaging pathways are activated, including oxidative stress and mitochondrial dysfunction.15–17 Recently, a direct linkage between β-amyloid and the mitochondria was defined, with β-amyloid binding to the mitochondrial protein AB alcohol dehydrogenase.18

The data in this study further underscore the parallels between CryABR120G cardiomyopathy and the amyloid-based neurodegenerative pathologies, as mitochondrial dysfunction appears to be an early event in the cardiac pathology, appearing by 6 to 8 weeks and before any overt changes in organ function can be detected. The biochemical, functional, and structural alterations resulted in a significantly compromised Pcr/ATP ratio in early adulthood (Figure 2) before overt functional deficits present. A number of processes could lead to early involvement of the mitochondria. Mitochondria are held in position and can be transported in the cytoplasm through their interactions with cytoskeletal components such as the microtubules and intermediate filaments.19 We have noted that disruption of the desmin network rapidly leads to alterations in mitochondrial positioning and structure,5 and similar observations have been made in striated muscle derived from the desmin knockout mice, with severe mitochondrial deficits presenting in both heart and skeletal muscle.9,13 We hypothesize that disturbance of the tight juxtaposition of the mitochondria over the interior of the sarcomere results in alterations in cellular metabolism. Our data are consistent with CryABR120G specifically associating with mitochondria through VDAC interaction early in the pathogenic process. The significance of the preferential association of CryABR120G versus the normal protein with VDAC is unclear.

Figure 6. Time lapse analysis of Δψ loss. RNC were transfected as in Figure 5. Five days after infection, cells were loaded with TMRE, treated with 100 nM of the uncoupler CCCP, and time lapse confocal microscopy begun. A, Sequential images for TMRE intensity for each group. B, Time course of normalized TMRE intensity. The average TMRE brightness from ≥30 myocytes from each plate was collected at the indicated times. To confirm the PTP dependency of the onset of TMRE loss, transfected cardiomyocytes were treated with CsA before exposure to CCCP. CsA significantly delayed Δψ dissipation. C, Time to 50% loss of TMRE intensity (*P<0.05 versus control or WT).
but raises the possibility that CryAB\textsuperscript{R120G} may have a direct impact on either VDAC or a mitochondrial protein associated with the PTP. What is clear is that mitochondrial dysfunction is one of the earliest detectable events in the development of R120G-mediated cardiomyopathy and appears to play a major role in the developing pathology. As early as 6 weeks, there is a significant reduction in complex I activity and mitochondrial respiration is significantly compromised. Mitochondrial permeability transition is clearly affected in CryAB\textsuperscript{R120G} transfected cardiomyocytes and precedes the increased levels of apoptotic markers.

The connections between amyloid deposition, mitochondrial dysfunction, and cell degeneration and death remain contentious. However, there are increasing data linking amyloidogenic proteins to mitochondrial toxicity. The exposure of isolated brain mitochondria to \( \beta \)-amyloid causes a decrease in mitochondrial enzyme activity, respiration, and membrane potential.\textsuperscript{20} \( \beta \)-Amyloid can activate PTP opening, resulting in mitochondrial swelling,\textsuperscript{21} a result consistent with our observations. Impaired function of complex I has also been linked to the development of Parkinson and Alzheimer diseases\textsuperscript{17,22,23} and in Down syndrome there are reduced levels of complex I in the cerebellum.\textsuperscript{24} In Parkinson disease, the proteins parkin and \( \alpha \)-synuclein, which are components of the abnormal aggregates (Lewy bodies) found in patient neurons, bind to one another in vitro and inhibition of the mitochondrial respiratory chain will increase incorporation of \( \alpha \)-synuclein into the aggregates in vitro.\textsuperscript{25} Finally, deficits in energy metabolism have been proposed as a primary pathogenic mechanism in Huntington disease, with elevated lactate levels being detected in the occipital cortex and basal ganglia.\textsuperscript{26} Ultrastructural analyses have demonstrated that mutant huntingtin appears to be present on neuronal mitochondrial membranes\textsuperscript{27} and can directly increase mitochondrial susceptibility to calcium-induced permeability transition, resulting in the release of cytochrome \( c \).\textsuperscript{28} These studies are consistent with the mechanisms that might be involved in CryAB\textsuperscript{R120G} pathogenesis, as our data show that CryAB\textsuperscript{R120G} expression leads to detectable amyloid formation in the cardiomyocytes\textsuperscript{8} and
mitochondrial dysfunction, which, in turn, could contribute to a more rapid amyloid accumulation and an inherently unstable feed-forward loop.

The release of cytochrome c is a well-defined mechanism for activation of apoptosis and, in the last few years, the potential importance of apoptosis in heart failure has been defined.\textsuperscript{29–32} Wencker et al\textsuperscript{33} established a causal role by showing that very low levels of myocyte apoptosis were sufficient to cause a lethal, dilated cardiomyopathy. In a retrospective study on 33 patients who had died of acute myocarditis, cardiomyocyte apoptosis was identified as a common mechanism of myocardial damage with significantly more apoptotic cardiomyocytes present in patients who had died from progressive heart failure compared with those who died suddenly from cardiac arrest.\textsuperscript{33} Cardiomyocytes transfected with CryAB\textsuperscript{R120G} show striking activation of both early and late apoptotic markers, confirming the potential of an acute response on mutant CryAB expression. Significantly, we also found high levels of activated caspase-3 in the CryAB\textsuperscript{R120G} transgenic hearts in the later disease stages of progressive heart failure (Figure 4). Taking into consideration that the progression of cardiomyopathy and heart failure in CryAB\textsuperscript{R120G} mice occurs over a 5- to 7-month period, our model underscores the potential importance of apoptosis in progressive heart failure.

Our data point to the importance of a progressive pathology in the development of heart failure. It is clear that expression of CryAB\textsuperscript{R120G} has acute effects on cardiomyocyte mechanics, affecting contractility through as yet undefined mechanisms, although the accumulating aggregates could certainly play a physical role in attenuating normal cardiomyocyte contractile behavior. Alterations in contractility can be sensed by multiple mechanisms, resulting in global responses at the transcriptional and translational levels but we believe that a crucial aspect of the early pathology is linked to alterations in respiration. Mitochondria-sarcromere architecture is affected very early and complex I activity is significantly attenuated with reductions of 50% by 6 weeks, before alterations in cardiac function can be detected. This is rapidly followed by compromised PTP function and mitochondrial swelling. These become more severe over a period of 2 to 3 months, eventually leading to release of cytochrome c and activation of apoptosis.

Acknowledgments

This work was supported by National Institutes of Health grants HL69779, HL56370, HL074728, HH61638, HL52318, HL69799, HL60546, HL52318, HL60546, and HL56370 (Dr Robbins) and by an American Heart Association Fellowship (Dr Maloyan).

References

Desmin-related myopathy, a skeletal and cardiac myopathy characterized by abnormal accumulations of desmin and α-B crystallin within muscle fibers, can be caused by a missense mutation in the chaperone, α-B crystallin (R120G). Our laboratory confirmed that transgenic mice with cardiac-specific R120G expression developed dilated cardiomyopathy at maturity and died in early adulthood from heart failure. Other data point to the intriguing hypothesis that although DRM is a rare disease, its pathology shares some characteristics with a spectrum of neurodegenerative diseases, including cytoplasmic reactivity with a toxic pre-amyloid protein conformer. These conformers appear to be widespread in cardiomyocytes derived from human heart failure patients, underlying the potential generality of aspects of the DRM pathology. However, the mechanism leading to development of dilated cardiomyopathy in the R120G model is unclear. We show that alterations in mitochondria architecture and function appear acutely after R120G gene expression, before other detectable pathologies and compromised cardiac function develop. These changes led to cytochrome c leakage from the mitochondria, which can often trigger apoptosis. Indeed, activation of apoptotic processes was confirmed both in vivo and in an in vitro study, which showed that R120G expression disrupted mitochondrial membrane potential, activated opening of the mitochondrial permeability transition pore and initiated apoptosis. Further investigation of these mutant chaperone-induced pathways and their general relevance to heart disease and cardiac failure will help identify novel therapeutic targets that could be effective against heart disease caused by a diverse set of primary genetic or environmental etiologies.
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Circulation. 2005;112:3451-3461
doi: 10.1161/CIRCULATIONAHA.105.572552
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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