On Genetics of Dilated Cardiomyopathy and Transgenic Models
All Is Not Crystal Clear in Myopathic Hearts

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The 
B-crystallin protein, the predominant structural protein of the ocular lens, is a member of the small heat shock proteins that is also expressed abundantly in the heart and skeletal muscle. The 
B-crystallin was initially discovered in the vertebrate ocular lens and was dubbed crystallin because of its role in maintenance of lens transparency. It is also essential for maintenance of microtubular integrity in striated muscles. In the heart, as in the ocular lens, 
B-crystallin forms soluble multimers that function as chaperone molecules, facilitating protein folding and translocation. Thus, the principal function of 
B-crystallin protein is to prevent unfolding of cellular proteins damaged by all forms of stress. Ischemia and oxidative stress increase the expression of 
B-crystallin in the heart. In response to stress, intracellular kinases phosphorylate 
B-crystallin, leading to its translocation from the cytosolic pool to Z lines and intercalated disks. Translocated 
B-crystallin binds to the components of the intermediary filaments and cytoskeletal proteins, such as actin and desmin, and prevents their aggregation. The protective role of 
B-crystallin in the maintenance of cytoskeletal integrity has been confirmed in gene transfer studies in cultured cardiac myocytes and in transgenic mice. Overexpression of 
B-crystallin protects cardiac myocytes against apoptosis and reperfusion injury. Interest in 
B-crystallin has been heightened because of recent elucidation of the genetic basis of desmin-related myopathy (DRM), a familial muscular disorder characterized by skeletal myopathy, heart failure, conduction defect, and arrhythmias. Pathologically, DRM is characterized by the presence of protein aggregates containing desmin in the cytoplasm of striated muscles. For this reason, the initial genetic studies in families with DRM focused on identification of mutations in the desmin gene (DES). However, genetic heterogeneity of DRM was soon recognized and substantiated by the discovery of the R120G mutation in the CRYAB gene in patients with DRM and lens cataract. In this issue of Circulation Research, Wang et al describe a transgenic mouse model whereby cardiac-restricted expression of the 
B-crystallin–R120G protein leads to a phenotype similar to DRM in humans. The 
B-crystallin–R120G mice exhibit early mortality, aberrant desmin and 
B-crystallin aggregation in the heart, and cardiac hypertrophy and dysfunction. The observed phenotypes are similar to those reported in the mutant desmin transgenic mice, which emphasize the intricate interactions between 
B-crystallin and desmin. In contrast to the mutant protein, overexpression of wild-type 
B-crystallin in the heart did not produce a significant phenotype. This finding corroborates the results of studies in a previous 
B-crystallin transgenic mouse model and in transgenic mice overexpressing wild-type desmin. A striking feature of the 
B-crystallin–R120G mice is the high rate of premature death from congestive heart failure leading to 100% fatality at 32 weeks of age. In humans, the R120G mutation has been described only in a single large family with an apparently low rate of premature death. This apparent discrepancy may reflect the relatively higher than natural levels of the mutant protein in the hearts of transgenic mice. The effects of the excess mutant protein on survival could also explain the lower copy number and expression levels observed in the mutant transgenic mice compared with the wild-type mice, ie, conferring an embryonic survival disadvantage. Other factors, such as the genetic background, known to affect the phenotypic expression of cardiomyopathies in humans, also could account for the observed differences in survival rates. The mutant 
B-crystallin mice, despite having deposits of desmin/ 
B-crystallin aggregates in the heart, exhibited an enhanced +dp/dt at 3 months of age. The latter may reflect the presence of left ventricular hypertrophy, although it should be recognized that +dp/dt is a load-dependent index, which varies significantly from mouse to mouse. It is interesting that the initial description of the phenotype in the family with the 
B-crystallin R120G mutation was that of hypertrophic cardiomyopathy. Despite apparent dissociation of the contractile function and cardiac hypertrophy, it is likely that hypertrophy, as in other forms of cardiomyopathy, is a secondary phenotype because of impaired myocyte function and activation of stress-responsive transcription machinery.

The primary purpose of transgenesis is to develop models that provide opportunity to delineate the pathogenesis of the disease of interest so that new targets for treatment and prevention of human disease might be developed. In this regard, the report by Wang et al provides some insight into the pathogenesis of DRM and supports the results of previous in vitro cell culture studies. The results in transgenic mice suggest that the 
B-crystallin–R120G is less soluble and its expression leads to formation of protein aggregates,
comprised, at least, of desmin and αB-crystallin. Previous in vitro studies provide sparse information that could only partially explain the basis for the observed phenotype in mice. The R120G mutation affects a highly conserved amino acid and alters secondary, tertiary, and quaternary structure of αB-crystallin. As a result, the mutation increases susceptibility of αB-crystallin to heat-induced denaturation and reduces its chaperone activity and the ability to prevent filament-filament interaction. 17,16 The precise mechanisms by which the mutant αB-crystallin–R120G leads to deposits of aggregates in the myocardium and DRM are unknown. Similarly, regulation of chaperone activity of αB-crystallin, the target motifs, and the substrate binding sites remain to be explored. Factors that determine the solubility of the αB-crystallin, the basis for the formation of aggregates, and their complete composition are also unknown. In addition, the effects of exercise, pH, temperature, mechanical stress, ischemia, and oxidative stress on the solubility and formation of aggregates require additional studies. The effects of the mutation on oligomerization, autophosphorylation, phosphorylation by stress-responsive kinases, nuclear translocation, and chaperone activity of αB-crystallin require additional investigation. Furthermore, target proteins affected by the altered chaperone activity of the mutant αB-crystallin and the impact of the mutation on the affinity of the αB-crystallin for cytoskeletal proteins are not fully understood. It is expected that the αB-crystallin–R120G mouse model will provide an opportunity to decipher some aspects of the molecular pathogenesis of DRM caused by the mutant αB-crystallin–R120G protein. Identification of the CRYAB as a causal gene for dilated cardiomyopathy (DCM) is also in accord with the notion that the primary defect in DCM is the integrity of the cytoskeleton, as initially proposed after identification of the first mutation in cardiac α-actin in patients with familial DCM.18 It is now clear that mutations in a variety of proteins with diverse structure and function can lead to DCM (Table). A unifying theme has emerged, which suggests that DCM occurs as a consequence of a common functional defect in the integrity of the cytoskeleton. Impaired function of the cy-
toskeleton, as a result of mutations in its primary components, intermediary filaments, or the supporting proteins, could lead to cardiac dilatation and heart failure. The αB-crystallin is considered essential for maintaining the integrity of the cytoskeleton. Therefore, delineation of the mechanism by which the mutant αB-crystallin loses its ability to support the cytoskeleton and causes DRM could provide significant insight into the pathogenesis of heart failure from all causes.

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References


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