Familial Hypertrophic Cardiomyopathy Mutations in the Regulatory Light Chains of Myosin Affect Their Structure, \( \text{Ca}^{2+} \) Binding, and Phosphorylation*

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The effect of the familial hypertrophic cardiomyopathy mutations, A13T, F18L, E22K, R58Q, and P95A, found in the regulatory light chains of human cardiac myosin has been investigated. The results demonstrate that E22K and R58Q, located in the immediate extension of the helices flanking the regulatory light chain Ca\(^{2+}\) binding site, had dramatically altered \( \text{Ca}^{2+} \) binding properties. The \( K_c \) value for E22K was decreased by ~17-fold compared with the wild-type light chain, and the R58Q mutant did not bind \( \text{Ca}^{2+}\). Interestingly, \( \text{Ca}^{2+} \) binding to the R58Q mutant was restored upon phosphorylation, whereas the E22K mutant could not be phosphorylated. In addition, the \( \alpha \)-helical content of phosphorylated R58Q greatly increased with \( \text{Ca}^{2+} \) binding. The A13T mutation, located near the phosphorylation site (Ser-15) of the human cardiac regulatory light chain, had 3-fold lower \( K_c \) than wild-type light chain, whereas phosphorylation of this mutant increased the \( \text{Ca}^{2+} \) affinity 6-fold. Whereas phosphorylation of wild-type light chain decreased its \( \text{Ca}^{2+} \) affinity, the opposite was true for A13T. The \( \alpha \)-helical content of the A13T mutant returned to the level of wild-type light chain upon phosphorylation. The phosphorylation and \( \text{Ca}^{2+} \) binding properties of the regulatory light chain of human cardiac myosin are important for physiological function, and alteration any of these could contribute to the development of hypertrophic cardiomyopathy.

There is substantial evidence that myosin regulatory light chains (RLC)\(^1\) play a primary regulatory role in scallop and smooth muscle contraction, but their functional role in mammalian striated (skeletal and cardiac) muscle contraction is unclear. RLC, together with the essential light chain, stabilizes the 8.5-nm-long \( \alpha \)-helical neck of the myosin head, with the N terminus of RLC wrapped around the heavy chain (1). Smooth muscle contraction is initiated by RLC phosphorylation with a \( \text{Ca}^{2+}\)-calmodulin-activated myosin light chain kinase (MLCK) (2, 3). However, in skeletal and cardiac muscle, RLC phosphorylation does not activate contraction but appears to play a modulatory role (4). It was shown that RLC phosphorylation increased the \( \text{Ca}^{2+} \) sensitivity of force in skinned skeletal (5–7) and cardiac (8) muscle fibers. In the human heart, several RLC isoforms are expressed (9, 10) preferentially in the atrium and in the ventricle. Recent studies have revealed that the ventricular RLC is one of the sarcomeric proteins associated with familial hypertrophic cardiomyopathy (FHC) (11, 12). FHC is an autosomal dominant disease, characterized by left ventricular hypertrophy, myofibrillar disarray, and sudden death. It is caused by missense mutations in various genes that encode for \( \beta \)-myosin heavy chain (13), myosin-binding protein C (14), ventricular RLC and essential light chain (11, 12, 15), troponin T (16), troponin I (17), \( \alpha \)-tropomyosin (18), actin (19), and titin (20). Depending on the affected gene, and the site of the mutation, FHC has variable presentation with regard to its degree and severity and the extent of myocardial disarray. The clinical manifestations of FHC range from benign to severe heart failure and to sudden cardiac death. The best characterized clinical cases include patients with \( \beta \)-myosin heavy chain mutations who have a high level of cardiac hypertrophy and those with troponin T mutations who have less hypertrophy, but a higher incidence of sudden cardiac death in young adults. The first three identified mutations in the RLC (A13T, E22K, and P95A) were shown to be associated with a particular subtype of cardiac hypertrophy defined by mid-left ventricular obstruction (11). Two other RLC mutations (F18L and R58Q), identified by Flavigny et al. (12), were associated with a typical form of hypertrophic cardiomyopathy, which causes increased left ventricular wall thickness and abnormal electrocardiograph findings with no mid-cavity obliteration.

The three-dimensional structure of the RLC demonstrates the close proximity of FHC mutations to either the phosphorylation site of RLC (Ser-15) or the \( \text{Ca}^{2+} \) binding site (amino acids 37–48). Because of this distinctive arrangement of the FHC mutations within the RLC, it was of interest to study their effect on the phosphorylation properties of human cardiac RLC (HCRLC) as well as their effect on \( \text{Ca}^{2+} \) binding. We have also investigated how these FHC mutations influence the secondary structure of the HCRLC as well as the combined effects of phosphorylation and \( \text{Ca}^{2+} \) binding on their structure. We demonstrate that both processes, phosphorylation and \( \text{Ca}^{2+} \) bind-
ing, are significantly altered by the FHC mutations and their effect depends upon the specific location of the missense mutation. The alterations in contractility that would result from these mutations are not known at present, and it is therefore not possible to know precisely how they might trigger the hypertrophic process. It is likely, however, that such a response would be part of a direct compensatory and/or adaptive mechanism of the heart to maintain normal cardiac function. Assessing the mechanism by which FHC mutations alter RLC function will lead to a better understanding of the physiological role of RLC in the regulation of cardiac muscle contraction.

MATERIALS AND METHODS

Mutation, Expression, and Purification of Wild-type HCRLC and the FHC Mutants—The cDNA for wild-type HCRLC was cloned by reverse transcription-polymerase chain reaction using primers based on the published cDNA sequence (GenBank accession no. A02076/86) and standard methods (21). The FHC RLC mutants: A13T, F18L, E22K, R58Q, and P95A, were generated using overlapping sequential polymerase chain reaction (21). Wild-type and mutant cDNAs were constructed with an NcoI site at the N-terminal ATG and a BamHI site following the stop codon to facilitate ligation into the NcoI-BamHI cloning site of the pET-3d (Novagen) plasmid vector and transformation into DH5α cloning host bacteria for expression of the cDNAs of the wild-type HCRLC and the FHC mutants. The cDNAs of these proteins were transformed into BL21 expression host cells and protein expressed in large (16 liters) cultures.

Expressed proteins were purified using a Q-Sepharose column followed by a DE-52 column, both equilibrated with 2 m NaCl, 0.1 m Tris-Cl, 1 m dithiothreitol, 0.01% NaN₃, pH 7.5. The proteins were eluted with a 1000-ml salt gradient of 0–300 mM KCl. The elution profiles from both the Q-Sepharose and the DE-52 columns for wild-type (WT) HCRLC and A13T occurred in the range of 160–200 mM KCl, for F18L, R58Q, and P95A between 210–250 mM KCl, and for E22K between 230 and 280 mM KCl. The final purity of the proteins was tested using 15% SDS-PAGE.

Phosphorylation of Wild-type HCRLC and Its FHC Mutants—The proteins were phosphorylated with Ca²⁺−calmodulin activated myosin light chain kinase (MLCK) in a solution containing: 50 μM protein (in 20 mM imidazole, 50 mM NaCl, 0.1 mM PMSF, pH 7.5), 0.1 mM NADPH, 0.5 mM MgCl₂, 5 mM ATP. A catalytically active truncated fragment of the rabbit skeletal muscle MLCK was used in this study (22). The MLCK, missing the first 256 amino acids, was expressed in Sf9 cells infected by a recombinant virus (23). The phosphorylation reaction was carried out for 2 h at room temperature or overnight on ice. After phosphorylation the proteins were purified using a Q-Sepharose column (conditions as described above). The level of phosphorylation was measured using 8% urea-PAGE (24).

Flow Dialysis—Flow dialysis was performed in a solution of 100 mM KCl, 20 mM imidazole, pH 7.0 (22 °C). All proteins were equilibrated in this buffer prior to the measurements. The flow dialysis experiments were performed according to Colowick et al. (25) with modifications. Briefly, the upper chamber of the apparatus containing the protein and the labeled substrate (³⁵Ca²⁺) was separated by a membrane from the lower chamber. The buffer was pumped through the lower chamber at a constant rate of 1.5 ml/min. The upper chamber was first equilibrated with 0.4 ml of buffer for 15 min, followed by the protein (0.4 ml) for 5 min. After adding the Ca²⁺−—an equilibrium was attained by flowing buffer through the lower chamber for 5 min. After steady state was reached, unlabeled substrate (Ca²⁺) was added at regular intervals and in varying concentrations. Fractions were collected every 30 s, and the effluent was sampled for measurement of radioactivity. The specific radioactivity of the Ca²⁺− used in the experiment was 12–16 mCi/mg (from PerkinElmer Life Sciences), and 2 μCi of Ca²⁺− per experiment gave sufficient radioactivity in the dialysate for accurate measurements. Data were presented using Scatchard analysis (26, 27).

Effect of Phosphorylation—Phosphorylation of HCRLC-WT with the cell-free fraction of MLCK decreased its Ca²⁺ binding affinity by 7.4-fold ($K_{Ca} = 9.0 ± 0.6 × 10^{-5}$ M⁻¹, Fig. 3A and Table I, n = 3, p < 0.001). However, two other FHC mutants, E22K and R58Q, dramatically changed the RLC's Ca²⁺ binding properties in various ways (Figs. 3 and 4). The most dramatic effect was observed for the E22K mutant. This substitution prevented the E22K mutant from becoming phosphorylated. Even a 20-fold increase of the enzyme/substrate ratio and longer incubation time did not result in phosphorylated E22K (Fig. 4, lane 5). As

RESULTS

To study the effects of FHC mutations on the phosphorylation, Ca²⁺− binding and the secondary structure of HCRLC, we have cloned and expressed the WT and five FHC mutants of HCRLC: A13T, F18L, E22K, R58Q, and P95A. Fig. 1A presents the amino acid sequence of human ventricular RLC and all the known specific missense mutations that have been associated with FHC. The Ca²⁺ binding (residues 37–48) and the phosphorylation sites (Ser-15) are also illustrated. Fig. 1B demonstrates the three-dimensional representation of the HCRLC derived from the crystal structure of chicken skeletal myosin S1 (1). The N-terminal region of HCRLC containing the phosphorylation site and two of the FHC mutations, A13T and F18L, is not shown since this region of the RLC was unresolved in the reported crystal structure (1). The three-dimensional representation of the HCRLC (Fig. 1B) suggests that the mutations A13T, F18L, E22K, and P95A are located in close proximity to the phosphorylation site of RLC (Ser-15), whereas the E22K and R58Q mutations occur in the immediate extension of the helices flanking the Ca²⁺− binding loop.

$K_{Ca} = 6.67 ± 0.21 × 10^{-5}$ M⁻¹ (average of n = 3 flow dialysis experiments ± S.D.). The Scatchard plots presented here are representative of one flow dialysis experiment, while the affinity constants demonstrated in Table I are the average of 2–4 flow dialysis experiments ± S.D. Three of the FHC mutations: A13T, F18L, and P95A, decreased their Ca²⁺− binding affinity 3-fold compared with HCRLC-WT (Table I, n = 3, p < 0.003). However, two other FHC mutants, E22K and R58Q, dramatically changed the RLC's Ca²⁺ binding properties. The $K_{Ca}$ value decreased by ~17-fold for the E22K mutant (Fig. 2 and Table I, n = 3, p < 0.001), whereas the R58Q mutation completely impaired Ca²⁺− binding (Table I).
shown in Fig. 4, the gel migration of the E22K mutant was slower than HCRLC-WT, F18L, and R58Q mutants, due to the Glu → Lys replacement that resulted in an additional positive charge of the E22K protein. The E22K mutation changes the isoelectric point of HCRLC from pI = 5.49 to pI = 5.10; therefore, the nonphosphorylated E22K migrates slower than the nonphosphorylated wild-type HCRLC. Accordingly, the phosphorylated forms of HCRLC-WT and its FHC mutants migrated faster than the nonphosphorylated ones due to the acidic phosphate group attached to Ser-15 of the phosphorylated proteins (Fig. 4). A large effect of phosphorylation was also observed for the A13T mutation. As shown in Fig. 1, this mutation is located next to Ser-15, the phosphorylation site of HCRLC. Phosphorylation of A13T resulted in a large increase (KCa value increased from 2.06 ± 0.23 × 10^5 M^-1 to 13.27 ± 0.24 × 10^5 M^-1) in its Ca^{2+} binding affinity compared with nonphosphorylated A13T (Fig. 3B and Table I, n = 3, p = 0.016). Interestingly, phosphorylated A13T demonstrated a 15-fold greater affinity for Ca^{2+} than phosphorylated HCRLC-WT, whereas nonphosphorylated A13T bound Ca^{2+} with a 3-fold lower affinity than nonphosphorylated-WT. No effect of phosphorylation on Ca^{2+} binding to F18L was observed (Table I, n = 3). However, there was an interesting effect of phosphorylation on Ca^{2+} binding to the R58Q mutant. This mutant did not bind Ca^{2+} in the nonphosphorylated state but did bind Ca^{2+} when phosphorylated (KCa = 3.04 ± 1.02 × 10^5 M^-1) (Table I).

Secondary Structure of HCRLC and Its FHC Mutants—Far-UV CD spectroscopy was used to detect changes in the secondary structure of the HCRLC-WT and all of the FHC mutants are listed in Table I. Flow dialysis was performed in the solution of 100 mM KCl, 20 mM imidazole, pH 7.0, and 2 μCi of ^45Ca^{2+}/experiment.
phosphorylation on HCRLC-WT and its FHC mutants. Data are the average of 2–4 experiments \((n)\), each consisting of 10 scans. The variation in the \(\alpha\)-helical content of the HCRLC-WT and its FHC mutants was below 2% for the individual experiments. As shown in Fig. 5A and Table II, the \(\alpha\)-helical content of HCRLC-WT and its FHC mutants ranged from 18% to 29%, with the highest for A13T. Replacement of the alanine residue with threonine (A13T) increased the \(\alpha\)-helical content of HCRLC from 18% \((n = 4)\) to 29% \((n = 2)\). This significant effect of the FHC mutation on the secondary structure of the HCRLC \((p < 0.01)\) was quite surprising since alanine has a high potential to form \(\alpha\)-helix. The second significant change was brought about by the E22K mutation, which resulted in an increase in the \(\alpha\)-helical content of HCRLC from 18% to 24% \((n = 2, p < 0.01)\). All other FHC mutations of HCRLC did not alter its \(\alpha\)-helical content in the apo-state (Fig. 5A and Table II).

**Effect of Phosphorylation—Phosphorylation of HCRLC-WT did not change its \(\alpha\)-helical content or the binding of Ca\(^{2+}\) to the phosphorylated form (Table II, \(n = 4\)). Only Ca\(^{2+}\) binding to nonphosphorylated HCRLC-WT had increased \(\alpha\)-helical content (23%, Table II, Fig. 5B). No change in \(\alpha\)-helix was observed for the phosphorylated F18L and R58Q mutations in the apo-state. However, a dramatic effect of phosphorylation was observed for the A13T mutant, whose \(\alpha\)-helical content was decreased from 29% to 19% \((n = 2, p < 0.01, Table II, Fig. 5C)\). The phosphorylated A13T mutant had the same \(\alpha\)-helical content as HCRLC-WT in both phosphorylated and nonphosphorylated states (Table II). Interestingly, the effect introduced by the FHC mutation (replacement of the alanine with threonine) was reversed by the phosphorylation of the mutant (decrease in \(\alpha\)-helix from 29% to 19%). As mentioned above, the R58Q mutation had the same \(\alpha\)-helical content before and after phos-
phorylation; however, Ca\textsuperscript{2+}\ binding only occurred with the phosphorylated form and this increased its \(\alpha\)-helical content from 20% to 28% (\(n = 2\), \(p < 0.01\), Table II).

**DISCUSSION**

This study investigates the effects of FHC mutations in myosin RLC on Ca\textsuperscript{2+}\ binding, phosphorylation, and secondary structural properties.

Since the ventricular myosin RLC belongs to the superfamily of EF-hand Ca\textsuperscript{2+}\ binding proteins, it was of interest to investigate the effect of the FHC mutations on its Ca\textsuperscript{2+}\ binding properties. Unlike other EF-hands, RLC contains only one Ca\textsuperscript{2+}\ binding EF-hand domain, between amino acids 37 and 48.

We have studied all known FHC RLC mutants with special attention to two mutations, E22K and R58Q, located in the immediate extension of the helices flanking the HCRLC Ca\textsuperscript{2+}\ binding site. Indeed, whereas A13T, F18L, and P95A mutants decreased the Ca\textsuperscript{2+}\ affinity of RLC by 3-fold, the E22K and R58Q mutants had even greater reduction in Ca\textsuperscript{2+}\ affinity compared with HCRLC-WT. The \(K_{\text{Ca}}\) value for E22K was decreased by 17-fold, and the R58Q mutant did not bind Ca\textsuperscript{2+}\ at all. Interestingly, Ca\textsuperscript{2+}\ binding to the R58Q mutant was restored upon phosphorylation while the E22K mutant could not be phosphorylated. Even a 20-fold increase of the MLCK-calmodulin concentration did not result in phosphorylation of the E22K mutant (Fig. 4).

The R58Q mutation that had Ca\textsuperscript{2+}\ binding completely eliminated, was quite surprising since other EF hand Ca\textsuperscript{2+}\-binding proteins, e.g. troponin C, calmodulin, contain the Gln residue (and not Arg) in the equivalent position in the helix C-terminal of the Ca\textsuperscript{2+}\ binding site. The arginine residue of HCRLC, however, is very conserved across species and a wide spectrum of other RLCs contains the Arg residue in this position (Fig. 6). It would be interesting to determine whether substitution of Arg to Gln in these RLCs would also result in the inactivation of their Ca\textsuperscript{2+}\ binding site in the nonphosphorylated state.

Interestingly, phosphorylation of the R58Q mutant at Ser-15 restored the Ca\textsuperscript{2+}\ binding site (Table I). The mechanism of this intriguing observation is not quite clear. Perhaps the extra negative charge from the phosphate group of the HCRLC N terminus changes the conformation of the Ca\textsuperscript{2+}\ binding site itself and/or the region flanking the Ca\textsuperscript{2+}\ binding loop, containing the R58Q residue. It is also possible that these two important regions of RLC, the Ca\textsuperscript{2+}\ binding and the phosphorylation sites, are communicating with each other in an allosteric manner.

**TABLE II**

| Protein        | Apo | + Ca\textsuperscript{2+} | + P | + P + Ca\textsuperscript{2+} |
|----------------|-----|--------------------------|-----|-------------------------------|
| HCRLC-WT       | 18% | 23                       | 18  | 18                            |
| A13T           | 29  | 25                       | 19  | 18                            |
| F18L           | 24  | 24                       | 22  | 21                            |
| E22K           | 24  | 20                       |     |                               |
| R58Q           | 20  | 22\textsuperscript{*}    | 20  | 28                            |
| P95A           | 19  | 23                       | ND  | ND                            |

* No Ca\textsuperscript{2+}\ binding as judged by flow dialysis.

This study investigates the effects of FHC mutations in myosin RLC on Ca\textsuperscript{2+}\ binding, phosphorylation, and secondary structural properties.

At 195–250 nm with a bandwidth of 1 nm. Mean residue ellipticity ([\(\theta\])\textsubscript{MRE} in degrees cm\textsuperscript{2}/dmol) for spectra was calculated using the following equation: [\(\theta\])\textsubscript{MRE} = [\(\theta\])/10\(\theta\)Cr\textsubscript{}\ltext{}\textsuperscript{2}, where [\(\theta\]) is the measured ellipticity in millidegrees, Cr\textsubscript{}\textsuperscript{2} is the mean residue molar concentration, and \(l\) is the path length in cm.
ner. This was also observed for the E22K mutation with a greatly reduced Ca\(^{2+}\) affinity that could prevent phosphorylation. It is worth mentioning that the glutamic acid that follows the phosphorylation site of RLC and precedes its Ca\(^{2+}\) binding site, is also conserved among species (Fig. 6). Moreover, the substitution of the positively charged Lys for the acidic Glu residue resulted in a significant increase in \(\alpha\)-helical content of the E22K mutant compared with HCRLC-WT (Table II). All these changes induced by the E22K mutation are most likely affecting the interaction of the mutated light chain with the heavy chain of myosin. One could speculate that this mutation, which eliminates phosphorylation of the protein and reduces its affinity for Ca\(^{2+}\), alters working cross-bridges during contraction and may contribute to the development of hypertrophy in the human heart (34).

As mentioned above, the three-dimensional representation of the HCRLC (Fig. 1B) suggests that mutations A13T, F18L, E22K, and P95A are located in close proximity to the phosphorylation site of HCRLC. Thus, any alterations affecting the structure and/or sequence near this site would be expected to influence the phosphorylation properties of the HCRLC and/or the relationship between phosphorylation and Ca\(^{2+}\) binding. The first mutation located near the phosphorylation site (Ser-15, A13T, resulted in 3-fold decrease in the \(K_{\text{ca}}\) value, while phosphorylation of this mutant caused an additional 6-fold increase in Ca\(^{2+}\) affinity. The \(K_{\text{ca}}\) value for phosphorylated A13T was 15 times larger than phosphorylated HCRLC-WT. Therefore, the consequences of the FHC mutation (A13T) were most profound in conditions where the protein became phosphorylated. Thus, these results clearly suggest a link between phosphorylation and Ca\(^{2+}\) binding to RLC plays a key role in the working heart (35, 39). One could speculate that both of these processes may operate as adaptive and/or protective mechanisms to either attenuate the effect of the FHC mutations and/or improve performance of the working muscle. Alterations introduced by the FHC mutations most likely interfere with the interaction of the RLC with the heavy chain of myosin (40) and affect the function of myosin cross-bridges during force generation (41). The region of the myosin heavy chain that contains the RLC has been postulated to undergo conformational changes that are important for working muscle (42, 43). The motions of this region of the myosin head were predicted by crystallographic models (42, 44) and studied further by fluorescence polarization spectroscopy (45). It was demonstrated that, during active contraction, the RLC binding domain of the myosin head undergoes repetitive conformational changes (tilt and twist) and therefore may play an active role during force generation in muscle. Therefore, alterations introduced by the FHC mutations could interfere with the physiological function of the RLC and contribute to malfunctioning of the human heart. Patients with the FHC RLC mutations have developed a phenotype of hypertrophic cardiomyopathy, without sudden death (11, 12). Our results suggest that phosphorylation and Ca\(^{2+}\) binding to HCRLC may simultaneously act to protect and attenuate the negative physiological consequences of the FHC mutations. Further work, in progress in our laboratory, is aimed at determining the physiological consequences of these mutations.

### REFERENCES

1. Rayment, I., Rypniewski, W. R., Schmidt-Base, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelman, D. A., Wesenberg, G., and Holden, H. M. (1993) Science 261, 50–58
2. Sobieszek, A. (1977) Eur. J. Biochem. 73, 477–483
3. Somlo, A. P., and Somlo, A. V. (1994) Nature 372, 231–236
4. Sweeney, H. L., Bowman, B. F., and Stull, J. T. (1993) Am. J. Physiol. 264, C1085–C1096
5. Tzauon, P. T., Stull, J. T., and Traugh, J. A. (1992) Eur. J. Biochem. 192, 205–209
6. Metzger, J. M., Greaser, M. L., and Moss, R. L. (1989) J. Gen. Physiol. 93, 855–881
7. Szczesna, D., Zhao, J., and Potter, J. D. (1996) Biophys. J. 70, A380
8. Morano, I., Hofmann, F., Zimmer, M., and Ruegg, J. C. (1985) FEBS Lett. 189, 221–224
9. Morano, I., Hadicke, K., Grom, S., Koch, A., Schweeney, R. H., Bohm, M., Bartel, S., Erdmann, E., and Krause, E. G. (1994) J. Mol. Cell. Cardiol. 26, 361–368
10. Morano, I., Hadicke, K., Haase, H., Bohm, M., Erdmann, E., and Schaub, M. C.
11. Poetter, K., Jiang, H., Hassanzadeh, S., Master, S. R., Chang, A., Dalakas, M. C., Raymont, I., Sellers, J. H., Pananapazir, L., and Epstein, N. D. (1996) Nat. Genet. 13, 63–69
12. Flavigny, J., Richard, P., Isnard, R., Carrier, L., Charron, P., Bonne, G., Forissier, J. F., Desnoes, M., Dubourg, O., Kondjia, M., Schwartz, K., and Haque, B. (1998) J. Mol. Med. 76, 208–214
13. Geisterfer Lowrance, A. A., Kass, S., Tanigawa, G., Vosberg, H. P., McKenna, W., Seidman, C. E., and Seidman, J. G. (1990) Cell 62, 999–1006
14. Watkins, H., Conner, D., Thierfelder, L., Jarcho, J. A., MacRae, C., McKenna, W. J., Maron, B. J., Seidman, J. G., et al. (1995) N. Engl. J. Med. 332, 1058–1064
15. Kimura, A., Harada, H., Park, J. E., Nishi, H., Satoh, M., Takahashi, M., Hiroe, M., Marumo, F., and Kimura, A. (1999) FEBS Lett. 453, 105–114
16. Thierfelder, L., Watkins, H., MacRae, C., Lamas, R., McKenna, W., Vosberg, H. P., Seidman, J. G., and Seidman, C. E. (1994) Cell 77, 701–712
17. Uyeda, T. Q., Abramson, P. D., and Spudich, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4459–4464
18. Dominguez, R., Freyzon, Y., Trybus, K. M., and Cohen, C. (1998) Cell 94, 1163–1169
19. Corrie, J. E., Brandmeier, B. D., Ferguson, R. E., Trentham, D. R., Kendrick-Jones, J., Hopkins, S. C., van der Heide, U. A., Goldman, Y. E., Sabido-David, C., Dale, R. E., Criddle, S., and Irving, M. (1999) Nature 400, 425–430