Mavacamten, formerly known as MYK-461 is a recently discovered novel small-molecule modulator of cardiac myosin that targets the underlying sarcomere hypercontractility of hypertrophic cardiomyopathy, one of the most prevalent heritable cardiovascular disorders. Studies on isolated cells and muscle fibers as well as intact animals have shown that mavacamten inhibits sarcomere force production, thereby reducing cardiac contractility. Initial mechanistic studies have suggested that mavacamten primarily reduces the steady-state ATPase activity by inhibiting the rate of phosphate release of β-cardiac myosin-S1, but the molecular mechanism of action of mavacamten has not been described. Here we used steady-state and pre-steady-state kinetic analyses to investigate the mechanism of action of mavacamten. Transient kinetic analyses revealed that mavacamten modulates multiple steps of the myosin chemomechanical cycle. In addition to decreasing the rate-limiting step of the cycle (phosphate release), mavacamten reduced the number of myosin-S1 heads that can interact with the actin thin filament during transition from the weakly to the strongly bound state without affecting the intrinsic rate. Mavacamten also decreased the rate of myosin binding to actin in the ADP-bound state and the ADP-release rate from myosin-S1 alone. We, therefore, conclude that mavacamten acts on multiple stages of the myosin chemomechanical cycle. Although the primary mechanism of mavacamten-mediated inhibition of cardiac myosin is the decrease of phosphate release from β-cardiac myosin-S1, a secondary mechanism decreases the number of actin-binding heads transitioning from the weakly to the strongly bound state, which occurs before phosphate release and may provide an additional method to modulate myosin function.

Hypertrophic cardiomyopathy (HCM) is one of the most prevalent forms of heritable cardiovascular disorders, affecting approximately 1 in 500 individuals (1). Clinical pathology of HCM is characterized by thickening of the left ventricular wall, decreased left ventricular cavity volume, and diastolic dysfunction, with characteristically hyperdynamic contraction (2,3). More than 70% of all HCM cases can be attributed to mutations in a variety of sarcomeric proteins (4). One of the most prevalent sites of mutation can be found on the gene encoding myosin heavy chain 7 (MYH7), which encodes β-cardiac myosin heavy chain, the major myosin isoform found in the adult human heart (5). Myosins are molecular motors responsible for converting the chemical energy of ATP hydrolysis into the mechanical force necessary for such processes as cell division and migration, vesicle trafficking, and muscle contraction (6).

There has been extensive research conducted over the years to determine the mechanistic consequences of the various mutations of β-cardiac myosin. Although a vast amount of data had been gathered, there are inconsistencies and disagreements with regard to the effects of each mutation from a biochemical and biophysical perspective. Some mutations, such as the R403Q mutation, have shown decreases in the enzymatic activity of myosin (7), which was later shown to be largely dependent on the isoform of myosin being studied, whereas other studies have reported increases in the enzymatic activity, tension development, and/or increased in vitro filament sliding velocities of myosin (8). It has been previously reported that a direct correlation exists between the ATPase activity of β-cardiac myosin and the rate of force generation in cardiac fibers (9). We believe that modulation of this increased ATPase activity can help to return the hyperdynamic unit back to normal levels and thus alleviate the pathological phenotype.

This increase in enzymatic activity led us to postulate that a molecule that decreases the ATPase activity of β-cardiac myosin would improve the overall contractile properties of a diseased HCM heart. It was previously shown that this can be achieved using the small molecule inhibitor mavacamten (Fig. 1) in mouse models harboring multiple HCM mutations (10). In this work Green et al. (10) showed that mavacamten reduced the steady-state ATPase activity of permeabilized mouse cardiac myofibrils and basal bovine cardiac myosin subfragment-1 (S1), thereby affecting both the α (primary isoform in rodent hearts) and β (primary isoform in large animal hearts) isoforms of cardiac myosin. It was further determined that mavacamten inhibited the phosphate release rate of bovine cardiac myosin subfragment-1 (S1) without affecting the ADP release rate in an actin-activated state (10).

The purpose of this current work is to delve into the molecular mechanism of mavacamten and its effect on multiple types of myosin. This work will utilize systems of increasing complex-
Mechanistic study of mavacamten

![Chemical structure of mavacamten.](image)

Figure 1. Chemical structure of mavacamten.

ity to minimal systems to identify the minimal catalytic unit on
which mavacamten has an effect. Transient kinetic analysis was
performed to determine the mechanism by which mavacamten
acts. Green et al. (10) reported that the primary mechanism of
mavacamten is inhibition of the phosphate release rate; our
analysis suggests that another step in the chemomechanical
cycle of myosin is also affected and may explain the mechanism
in greater detail.

Results

Steady-state characterization of mavacamten

As previously reported (10), a small molecule screen was
conducted against the bovine cardiac myofibril system to
identify molecules that decreased the steady-state ATPase rate,
thereby reducing the ensemble force generation of the sarcomere.
Optimization of chemical matter from this screen led to the
development of mavacamten. Myofibrils generated from
human and bovine cardiac tissues as well as rabbit psoas (fast
skeletal) were used to determine pCa50 values for each system.
Concentration-dependent changes in myofibril ATPase at their
respective pCa50 values show a similar IC50 for bovine and
human systems, both of which have a 4-fold potency enhance-
ment over fast skeletal myofibrils (Table 1). Mavacamten was
found to have an IC50 value of 0.49 μM in the bovine system,
0.71 μM in the human system, and 2.14 μM in the rabbit system,
indicating selectivity of >4-fold for cardiac myosin (Fig. 2).
Analysis of the curve fit for these samples also shows a calcu-
lated Hill coefficient of ~2 for both the rabbit skeletal and
human cardiac myofibril systems. This deviation may be due to
the heterogeneous nature of the protein preparation for each
sample and potential variations in their respective prepara-
tions. A concentration-dependent response of mavacamten
was also run in the in vitro motility (IVM) assay to assess its
effect on actin filament sliding velocities. Cardiac heavy mero-
myosin (HMM) on a glass surface was used to propel actin
filaments in the presence of ATP and compound or DMSO. It
was found that mavacamten slowed the sliding velocity of actin
filaments in a concentration-dependent manner with a calcu-
lated IC50 of 0.587 ± 0.149 μM, confirming the effect of mava-
camten on a soluble form of double-headed myosin (Fig. 3).
Notably, the movement of these filaments was more sporadic
and “search-like” with mavacamten consistent with a mech-
anism that decreases the phosphate release rate of myosin and,
therefore, entry into the strongly bound state measured by
IVM.

To further elucidate the effect of mavacamten and determine
the minimal system in which mavacamten elicits a response,
concentration-dependent response measurements were per-
formed on a system utilizing myosin S1 generated from bovine
cardiac, rabbit psoas, and chicken gizzard tissues as well as a
recombinant form of human β-cardiac myosin and purified
bovine cardiac actin (Fig. 4). Mavacamten was shown to
inhibit all systems with potencies in the same range as the
myofibril system, with the exception of smooth muscle myo-
sin derived from chicken gizzard (Table 2). These data, along
with concentration-dependent response measurements against
basal myosin (data not shown), allows us to deduce that mava-
camten exerts its effect via the myosin-S1 motor. It is noteworthy
that there is increased specificity between the cardiac and skeletal
isofoms (~10×) compared with the myofibril system (~4×),

Table 1

Mavacamten IC50 and Hill coefficient values for multiple myofibril
systems

| Myofibril type (pCa50)  | IC50 value | Hill coefficient |
|------------------------|-----------|-----------------|
| Bovine cardiac (6.25)  | 0.490 ± 0.027 | 1.1 ± 0.02 |
| Rabbit fast skeletal (6.00) | 2.14 ± 0.27 | 2.14 ± 0.06 |
| Human cardiac (6.00)   | 0.711 ± 0.099 | 2.04 ± 0.06 |

![Concentration-dependent response curve of mavacamten](image)

Figure 2. Concentration-dependent response curve of mavacamten (MYK-461) in multiple myofibril-based systems. Myofibrils were assayed at pCa50 values of 6.25, 6.00, and 6.00 for bovine cardiac (1 mg/ml), rabbit skeletal (0.25 mg/ml), and human cardiac (1 mg/ml) respectively. Final assay conditions contained 2% DMSO with varying concentrations of mavacamten. Data were normalized to a DMSO control for each myofibril type assayed and analyzed using a four parameter fit (GraphPad Prism) to determine IC50 values of mavacamten. DMSO control rates of 0.097 ± 0.0002 μM/s, 0.158 ± 0.01 μM/s, and 0.104 ± 0.005 μM/s were measured for bovine cardiac, rabbit skeletal, and human cardiac myofibrils, respectively.

![Mavacamten (MYK-461) decreased in vitro motility-sliding velocities](image)

Figure 3. Mavacamten (MYK-461) decreased in vitro motility-sliding velocities. The final in vitro motility buffer contained 2% DMSO with or without mavacamten at varying concentrations. Sliding velocities were normalized to DMSO only velocities. n = 3 for all data points. For each n, >500 filament trajectories were quantified, and the median velocity from each of the 3 data sets was averaged.

![Actomyosin](image)

Figure 4. Actomyosin.
possibly due to the increased complexity of the myofibril system and that mavacamten shows no activity against the smooth muscle isoform of myosin-S1. To answer an open question of whether mavacamten would exert the same activity on mutant myosin-S1 as compared with wild-type bovine and human protein, concentration-dependent response experiments were performed against five mutations known to be associated with HCM. Fig. 5A shows the response curves for the mutant forms of recombinant human cardiac myosin, and their respective IC50 values are given in Table 2. Mavacamten inhibits each of these mutants to varying degrees, with the greatest potency conferred against the G741R mutant, 0.653 μM, and lower potency against the R719W mutant, 1.31 μM. Given that the potency of mavacamten against the wild-type form of human cardiac myosin-S1 is 0.727 μM, these values are remarkably similar (Fig. 5B).

**Transient kinetic analysis of mavacamten**

To begin dissecting the mechanism by which mavacamten exerts its effect on cardiac myosin, we utilized presteady-state methods to interrogate multiple steps in the chemomechanical cycle of myosin (Fig. 6). As previously reported (10), mavacamten is known to inhibit the phosphate release rate (Fig. 6, step 5) of bovine cardiac myosin-S1 using the method of Brune et al. (11). We found that under single turnover conditions mavacamten inhibited the actin-associated phosphate release rate in a concentration-dependent manner with a maximal inhibition of 80% at the highest concentration tested (10 μM) and with an IC50 value of 1.85 μM (10). We have built upon this work to determine the effect of mavacamten on the recombinant human cardiac myosin-S1 construct. Mavacamten inhibited the human form of the enzyme in a concentration-dependent manner and to a similar extent as the bovine cardiac myosin-S1 system (Fig. 7). The IC50 value for the human construct was calculated to be 1.78 μM.

To further the mechanistic characterization of mavacamten, the ADP release rates were measured for the actin-associated (Fig. 6, step 7) and basal (myosin alone) (Fig. 6, step 8) states of bovine cardiac myosin-S1 (Fig. 8) using mant-ADP. Little difference was observed in the ADP release rate in an actomyosin system: DMSO and 20 μM mavacamten response rates of 128.9 ± 3.10 s⁻¹ and 132.2 ± 3.05 s⁻¹, respectively. Alternatively, ADP release rates in the basal system were found to be 0.2616 ± 0.0005 s⁻¹ and 0.1402 ± 0.0007 s⁻¹ for DMSO and 20 μM mavacamten.
Previously it was reported that mavacamten did not affect the dissociation of myosin from actin in the ADP-bound state (10). To determine the effect of mavacamten on the rate of actin association in a strongly bound state (Fig. 6, step 6), a single mix stopped-flow experiment was performed in which myosin in the ADP-bound state of S1 was mixed with varying concentrations of pyrene actin in the presence and absence of 20 \(\mu M\) mavacamten (Fig. 9). The binding rate was measured via a decrease in the fluorescent transient of pyrene actin. The data in Fig. 9 were plotted and fit by linear regression, and the second order rate constant was calculated to decrease by \(\sim 4\)-fold, with values of 0.01455 \(s^{-1}\) and 0.1402 \(s^{-1}\) for DMSO and mavacamten, respectively.

To determine the effect of mavacamten on the weak to strong transition (Fig. 6, step 4) we used the method previously reported by De La Cruz et al. (12) for measuring the transition of myosin V. The rate of transition was measured with varying concentrations of mavacamten, and it was found that the compound had no effect on the transition rate, which was \(\sim 25\) \(s^{-1}\) (Fig. 10). The amplitudes were compared to determine if there was an effect on the number of myosin heads in the transition state that could bind to actin filaments. It was observed that increasing amounts of mavacamten decreased the amplitude of pyrene quenching by cardiac myosin-S1 in the transition state by 43% at the highest concentration.
we have also confirmed this effect on recombinant human cardiac myosin-S1 in a concentration-dependent fashion, and of action of mavacamten.

Cardiac myosin-S1 to determine the more precise mechanism of action of mavacamten. The observation that mavacamten slowed steady-state ATPase kinetics as well as sliding state or phosphate release (16). The observation that mavacamten slowed steady-state ATPase kinetics as well as sliding state or phosphate release (16). However, it has been recently shown that sliding velocities in IVM may not be detachment-limited (gated by the ADP release rate) but also attachment-limited (gated by the entry into the strongly bound state or phosphate release) (16). The observation that mavacamten slowed steady-state ATPase kinetics as well as sliding velocities led us to perform stopped flow experiments to interrogate individual steps in the chemomechanical cycle of bovine cardiac myosin-S1 to determine the more precise mechanism of action of mavacamten.

Mavacamten reduces the phosphate release rate step in the chemomechanical cycle. But this may not be the only step of the cycle that is affected by mavacamten. Looking at the inhibitory effect of mavacamten in the in vitro motility assay, an argument can be made that ADP release may also be affected by the compound. When looking at the kinetic transients of ADP release in Fig. 8, we see that the ADP release rate of bovine cardiac myosin-S1 in an actin-associated state was not affected by mavacamten (Fig. 8B), but the basal (myosin alone) rate of ADP release was inhibited by ~50%. Furthermore, looking at the actin association rate of myosin-S1 in the ADP state, we saw a 4-fold reduction in the second-order rate constant for actin binding in the presence of mavacamten (Fig. 9). Taking these two parameters into account, we can see that decreased binding in a known strongly bound state can begin to describe the motility data. By decreasing the rate of ADP release to prime the myosin head to initiate another turnover, thereby another stroke, and decreasing the rate of binding in this strongly bound state, mavacamten may ultimately be taking heads out of the cycle and causing them to be “parked” into a state that is nonproductive in the chemomechanical cycle of myosin-S1.

This hypothesis was interrogated using single turnover kinetics and pyrene actin fluorescence to determine the weak to strong transition rate of myosin-S1 to actin. The data collected showed very little change in the rate of transition, 24–26 s⁻¹, but a marked difference in the amplitudes of the reaction comparing DMSO and 40 μM mavacamten. The representative traces shown in Fig. 10 show a >40% reduction in fluorescence amplitude, which has been shown to represent the number of heads bound during this transition (12). This is the first piece of evidence to show a direct effect of mavacamten on the binding of myosin-S1 to actin in the transition state and gives some clarity as to its mechanism of action.

**Mechanistic study of mavacamten**

**Discussion**

Because the discovery of mavacamten and the findings that showed encouraging effects in mice with a hypertrophic cardiomyopathy phenotype, we have begun experiments to deduce its mechanism of action on β-cardiac myosin. Steady-state characterization has taken us from discovery on the level of a complex myofibril to potency measurements on more purified systems, such as actomyosin and the myosin-S1 motor unit. Collectively, the data show that mavacamten reduced the ATPase rate of both the actin-associated and basal systems. Further findings showed that mavacamten is selective with respect to the β-cardiac isoform of myosin, with IC₅₀ values of 0.473, 0.727, 5.852, and >50 μM in the bovine cardiac, human cardiac, rabbit fast skeletal, and chicken gizzard smooth muscle actomyosin systems respectively (Fig. 4). An in vitro motility assay was also performed and showed that mavacamten has a very similar IC₅₀ compared with the purified actomyosin system.

Mavacamten showed similar IC₅₀ values (a measure of potency) in both assays. The velocity with which actin filaments move across the HMM-covered surface in the IVM assay is largely thought to be rate limited by the ADP release rate of the myosin motor (15), whereas the steady-state ATPase assay is rate-limited by phosphate release (13, 14). However, it has been recently shown that sliding velocities in IVM may not be detachment-limited (gated by the ADP release rate) but also attachment-limited (gated by the entry into the strongly bound state or phosphate release) (16). The observation that mavacamten slowed steady-state ATPase kinetics as well as sliding velocities led us to perform stopped flow experiments to interrogate individual steps in the chemomechanical cycle of bovine cardiac myosin-S1 to determine the more precise mechanism of action of mavacamten.

Mavacamten reduces the phosphate release rate step of bovine cardiac myosin-S1 in a concentration-dependent fashion, and we have also confirmed this effect on recombinant human β-cardiac myosin-S1, with an IC₅₀ of 1.78 μM (Fig. 7). This finding coupled with the effect of mavacamten on the mutant myosins also tested allows us to conclude that the primary mechanism of action for this compound is the inhibition of the phosphate release step in the chemomechanical cycle. But this may not be the only step of the cycle that is affected by mavacamten. Looking at the inhibitory effect of mavacamten in the in vitro motility assay, an argument can be made that ADP release may also be affected by the compound. When looking at the kinetic transients of ADP release in Fig. 8, we see that the ADP release rate of bovine cardiac myosin-S1 in an actin-associated state was not affected by mavacamten (Fig. 8B), but the basal (myosin alone) rate of ADP release was inhibited by ~50%. Furthermore, looking at the actin association rate of myosin-S1 in the ADP state, we saw a 4-fold reduction in the second-order rate constant for actin binding in the presence of mavacamten (Fig. 9). Taking these two parameters into account, we can see that decreased binding in a known strongly bound state can begin to describe the motility data. By decreasing the rate of ADP release to prime the myosin head to initiate another turnover, thereby another stroke, and decreasing the rate of binding in this strongly bound state, mavacamten may ultimately be taking heads out of the cycle and causing them to be “parked” into a state that is nonproductive in the chemomechanical cycle of myosin-S1.

This hypothesis was interrogated using single turnover kinetics and pyrene actin fluorescence to determine the weak to strong transition rate of myosin-S1 to actin. The data collected showed very little change in the rate of transition, 24–26 s⁻¹, but a marked difference in the amplitudes of the reaction comparing DMSO and 40 μM mavacamten. The representative traces shown in Fig. 10 show a >40% reduction in fluorescence amplitude, which has been shown to represent the number of heads bound during this transition (12). This is the first piece of evidence to show a direct effect of mavacamten on the binding of myosin-S1 to actin in the transition state and gives some clarity as to its mechanism of action.

**Experimental procedures**

**Protein preparation**

Cardiac myofibrils were prepared as previously described (17). Bovine cardiac tissue was harvested, placed immediately on wet ice, and shipped overnight, and the left ventricle and septum were dissected, frozen in liquid nitrogen, and stored at −80 °C. Human tissue was procured from BioReclamations IVT (New York), and myofibrils were prepared on the day of receipt. Cardiac and skeletal myosin S1 was prepared using a chymotryptic digestion of full-length myosin prepared from bovine cardiac left ventricle and rabbit psoas muscle, respectively (18). Bovine cardiac HMM was prepared according to Margossian and Lowey (18). Human cardiac myosin subfragment-1 was expressed in differentiated murine C2C12 myotubes using an adenovirus infection method (19). The recombinant protein utilized a 6× histidine tag on the essential light chain for initial purification on Ni²⁺-resin with further purification by anion exchange and size exclusion chromatography. All myofibril and myosin-S1 preparations were brought to 10% sucrose, snap-frozen in liquid nitrogen, and stored at −80 °C. Actin was prepared from a bovine cardiac acetone powder (Pel Freez Biologicals) according to the method of Spudich and

Figure 10. Weak to strong transition of myosin-ADP-Pᵢ binding to pyrene actin in the presence of 1 mM ADP. Single turnover conditions in double mix mode are shown. Myosin-S1 (0.5 μM final) and ATP (0.5 μM final) were mixed and aged for 2 s and then mixed with 0.5 μM pyrene actin and 1 mM ADP. Representative traces show that increasing concentrations of mavacamten (MYK-461) reduce the amplitude of pyrene quenching caused by fewer myosin heads interacting with the actin filament.
Mechanistic study of mavacamten

Watt (20). Pyrene actin was prepared according to the method of Criddle et al. (21).

Data analysis

All curve-fitting was conducted using GraphPad Prism 7.0. Data were processed or preprocessed using Microsoft Excel, SoftMax Pro (Molecular Devices), or KinetAsyst (TgK Scientific). All experiments were performed in triplicate, and the means and standard deviations were obtained from three independent experiments, with each performed in triplicate.

Steady-state characterization

ATPase measurements were conducted using a coupled enzyme system utilizing pyruvate kinase and lactate dehydrogenase. Unless otherwise stated, the buffer system used in all experiments was 12 mM Pipes, 2 mM MgCl₂, 1 mM DTT at pH 6.8 (PM12 buffer). All steady-state experiments were carried out at 20 °C using a SpectraMax 384Plus plate reader, and rates were recorded using the SoftMax Pro software package. For all steady-state experiments, data were collected in triplicate and averaged, with n = 3. The value for n refers to the number of individual experiments performed. All data analysis of the steady-state systems were conducted using GraphPad Prism.

Transient kinetic characterization

Transient kinetic experiments were performed using a stopped-flow apparatus (Hi-Tech Scientific, SF-61 DX2) to determine the effects of mavacamten on myosin association and dissociation from actin filaments (21, 22), phosphate (P_i) release (11, 23), and 2′-(or-3′)-O-(N-methylanthraniloyl)-ADP (mant-ADP/ATP) release by myosin (23). For each data point, transient traces were collected in triplicate and averaged for a single experiment, with 100–1000 filament trajectories, from different surfaces were analyzed, and the median velocities of these were averaged for a single n. Each data point shown is a combination of 3–4 individual experiments (n = 3–4, each containing 500–3000 filament tracks).

In vitro motility assay

After thawing HMM, inactive HMM heads were removed before performing the motility assay. Actin was polymerized by adding 25 mM KCl and 2 mM MgCl₂ and incubating for 30 min at 4 °C. HMM was combined with polymerized actin, and 2 mM ATP was then added to the actomyosin solution to release functional HMM heads from actin, and the sample was centrifuged at 350,000 × g for 15 min. The supernatant containing active HMM was used for experiments. Fluorescent actin filaments were made by combining 1 μM tetramethylrhodamine phalloidin with 1 μM actin in IVM assay buffer (25 mM Imidazole, pH 7.4, 25 mM KCl, 4 mM MgCl₂, 1 mM EGTA, 1 mM DTT) containing 1 mM ATP. Tetramethylrhodamine-phalloidin-labeled filaments were stored in the dark at 4 °C for up to 1 week.

Movement of F-actin filaments over HMM-coated surfaces was achieved using a modification to the method of Uyeda et al. (26). When used, MyoKardia compounds were added to the final motility buffer with a final DMSO concentration of 2%, a concentration that does not significantly affect actin sliding velocities (data not shown). Images were acquired on a Nikon Eclipse Ti microscope at a frame rate of 2 Hz with a 100× oil immersion objective. Rigorous experimental design ensured that data sets contained >95% moving filaments. Custom analysis software was created by VigeneTech, in which images were set to a threshold based on pixel intensity, filaments were identified, and trajectories were determined for each filament. Only filaments >2 μm were recorded. 3–4 movies, each with 100–1000 filament trajectories, from different surfaces were analyzed, and the median velocities of these were averaged for a single n. Each data point shown is a combination of 3–4 individual experiments (n = 3–4, each containing 500–3000 filament tracks).
Mechanistic study of mavacamten

Author contributions—R. F. K. conducted all steady-state and most transient kinetic experiments, analyzed the results, and wrote the manuscript. R. L. A. conducted the transient kinetic experiments and analyzed the results. S. R. B. I. conducted the IVM experiments, analyzed the data, and wrote the corresponding sections of the manuscript. Y. S. and A. S. S. synthesized mavacamten. H. M. R. provided guidance for the experiments needed to prepare the manuscript. All authors reviewed the results, provided comments, and approved the final version of the manuscript.

References

1. Maron, B. J., Gardin, J. M., Flack, J. M., Gidding, S. S., Kurosaki, T. T., and Bild, D. E. (1995) Prevalence of hypertrophic cardiomyopathy in a general population of young adults: echocardiographic analysis of 4111 subjects in the CARDIA study. coronary artery risk development in (young) adults. Circulation 92, 785–789
2. Wilson, W. S., Criley, J. M., and Ross, R. S. (1967) Dynamics of left ventricular emptying in hypertrophic subaortic stenosis: A cineangiographic and hemodynamic study. Am. Heart J. 73, 4–16
3. Stewart, S., Mason, D. T., and Braunwald, E. (1968) Impaired rate of left ventricular filling in idiopathic hypertrophic subaortic stenosis and valvular aortic stenosis. Circulation 37, 8–14
4. Ramaraj, R. (2008) Hypertrophic cardiomyopathy: etiology, diagnosis, and treatment. Cardiol. Rev. 16, 172–180
5. Gryf, N., Luedde, M., and Katus, H. A. (2011) Mechanisms of disease: hypertrophic cardiomyopathy. Nat. Rev. Cardiol. 9, 91–100
6. Krendel, M., and Mooseker, M. S. (2005) Myosins: tails (and heads) of functional diversity. Physiology 20, 239–251
7. Cuda, G., Fananapazir, L., Epstein, N. D., and Sellers, J. R. (1997) The in vitro motility activity of β-cardiac myosin depends on the nature of the β-myosin heavy chain gene mutation in hypertrophic cardiomyopathy. J. Muscle Res. Cell Motil. 18, 275–283
8. Tyska, M. J., Hayes, E., Giewat, M., Seidman, C. E., Seidman, J. G., and Warshaw, D. M. (2000) Single-molecule mechanisms of R403Q cardiac myosin isolated form the mouse model of familial hypertrophic cardiomyopathy. Circ. Res. 86, 737–744
9. Brenner, B., and Eisenberg, E. (1986) Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. Proc. Natl. Acad. Sci. U.S.A. 83, 3542–3546
10. Green, E. M., Wakimoto, H., Anderson, R. L., Evanchik, M. J., Gorham, J. M., Harrison, B. C., Henze, L., Kawa, R., Olsen, J. D., Rodriguez, H. M., Song, Y., Wan, W., Leinwand, L. A., Spudich, J. A., McDowell, R. S., Seidman, J. G., and Seidman, C. E. (2016) A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. Science 351, 617–621
11. Brown, M., Hunter, J. L., Corrie, J. E., and Webb, M. R. (1994) Direct, real-time measurement of rapid organic phosphate release using a novel fluorescence probe and its application to actomyosin subfragment 1 ATPase. Biochemistry 33, 8262–8271
12. De La Cruz, E. M., Wells, A. L., Rosenfeld, S. S., Ostap, E. M., and Sweeney, H. L. (1999) The kinetic mechanism of myosin V. Proc. Natl. Acad. Sci. 96, 13726–13731
13. Lionel, C., Iorga, B., Candau, R., Piroddi, N., Webb, M. R., Belus, A., Travers, F., and Barman, T. (2002) Evidence that phosphate release is the rate-limiting step on the overall ATPase of psoas myofibrils prevented from shortening by chemical cross-linking. Biochemistry 41, 13297–13308
14. Iorga, B., Candau, R., Travers, F., Barman, T., and Lioine, C. (2004) Does phosphate release limit the ATPases of soleus myofibrils? Evidence that (A)M-ADP-P states predominate on the cross-bridge cycle. J. Muscle Res. Cell Motil. 25, 367–378
15. Sato, M. K., Ishihara, T., Tanaka, H., Ishijima, A., and Inoue, Y. (2012) Velocity-dependent actomyosin ATPase cycle revealed by in vitro motility assay with kinetic analysis. Biophys. J. 103, 711–718
16. Brizendine, R. K., Alcala, D. B., Carter, M. S., Haldeman, B. D., Facemyer, K. C., Baker, J. E., and Cremo, C. R. (2015) Velocities of unloaded muscle filaments are not limited by drag forces imposed by myosin cross-bridges. Proc. Natl. Acad. Sci. U.S.A. 112, 11235–11240
17. Patel, B. G., Wilder, T., Solaro, R. J. (2013) Novel control of cardiac myofilament response to calcium by S-glutathionylation at specific sites of myosin binding protein C. Front. Physiol. 4, 336
18. Margossian, S. S., and Lowey, S. (1982) Preparation of myosin and its subfragments from rabbit skeletal muscle. Methods Enzymol. 85, 55–71
19. Deacon, J. C., Bloemink, M. J., Rezavandi, H., Geeves, M. A., and Leinwand, L. A. (2012) Identification of functional differences between recombinant human β and β cardiac myosin motors. Cell. Mol. Life Sci. 69, 2261–2277
20. Spudich, J. A., and Watt, S. (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. J. Biol. Chem. 246, 8466–8471
21. Cridle, A. H., Geeves, M. A., and Jeffries, T. (1985) The use of actin labelled with N-(1-pyrenyl)iodoacetamide to study the interaction of actin with myosin subfragments and troponin/troponin. Biochem. J. 232, 343–349
22. Cooper, J. A., Walker, S. B., and Pollard, T. D. (1983) Pyrene actin: documentation of the validity of a sensitive assay for actin polymerization. J. Muscle Res. Cell Motil. 4, 253–262
23. Gilbert, S. P., and Mackey, A. T. (2000) Kinetics: a tool to study molecular motors. Methods 22, 337–354
24. Radke, M. B., Taft, M. H., Stapel, B., Hiffler-Kleiner, D., Preller, M., Manstein, D. J. (2014) Small molecule-mediated refolding and activation of cardiac myosin motors. Elife 3, e01603
25. Kovacs, M., Tóth, J., Hetényi, C., Málnási-Csizmadia, A., and Sellers, J. R. (2004) Mechanism of blebbistatin inhibition of myosin II. J. Biol. Chem. 279, 35557–35563
26. Uyeda, T. Q., Kron, S. J., and Spudich, J. A. (1990) Myosin step size: estimation from slow sliding movement of actin over low densities of heavy meromyosin. J. Mol. Biol. 214, 699–710