Review Article

The mechanisms of cardiac myopathies, a kinetics approach: Leading review

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Introduction

The normal adult heart is a well maintained machine that has a mechanism for growth replacement of the sarcomere that is lost by natural degeneration. This process ensures the heart has the strength of contraction to function correctly giving blood supply to the whole body. Some of the force of contraction of the sarcomere is transmitted to its major protein titin where its strength results in unfolding of a flexible section and release of a growth stimulant. The origin of all the cardiomyopathies can be traced to errors in this system resulting from mutations in a wide variety of the sarcomeric proteins. Too much or chronic tension transfer to titin giving increased growth resulting in hypertrophic cardiomyopathy (HCM) and too little leading to muscle wastage, dilated cardiomyopathy (DCM). HCM can ultimately lead to sudden cardiac death and DCM to heart failure. In this paper I show (1) a collection of the tension/ATPase calcium dependencies of cardiac myofibrils that define the mechanism of Ca^{2+} cooperativity. (2) I then reintroduce the stress/strain relationship to cardiomyopathies. (3) I then review the cardiomyopathy literature that contains similar Ca^{2+} dependency data to throw light on the mechanisms involved in generation of the types of myopathies from the mutations involved. In the review of cardiomyopathy there are two sections on mutations, the first dealing with those disrupting the Ca^{2+} cooperativity, i.e. the Hill coefficient of activation, leading to incomplete relaxation in diastole, chronic tension, and increased growth. Secondly dealing with those where the Ca^{2+} cooperativity is not affected giving either increased or decreased tension transfer to titin and changes in sarcomere growth.

These considerations have confirmed that the inhibitory function of cardiac troponin-I (cTnI) is part of a concerted process with the regulatory cardiac myosin binding protein-C (cMyBP-C) to block the use of myosin light chain (MLC) bound magnesium adenosine triphosphate (MgATP) from being used as cross-bridge substrate until the magnesium bound is exchanged for calcium. This Ca^{2+} dependency added to the trigger troponin-C (cTnC) Ca^{2+} binding is the origin of the Ca^{2+} cooperativity (Figure 1).
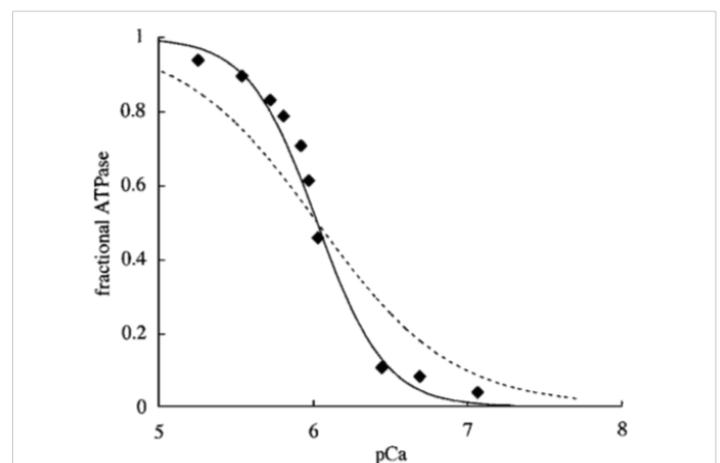

Figure 1: Ca^{2+} dependence of myofibrillar ATPase. Data points (◆) are reproduced, with permission, from Holroyde, et al. [1]. Lines represent best fits for 1:1 Ca^{2+} (dotted) and 2:1 Ca^{2+} (solid) binding schemes. Half maximal is 6.1 M. Smith, et al. [2].

The Ca²⁺ activation of the myofibrils is cooperative

The conclusion is that two Ca²⁺ binding sites are occupied cooperatively for activation of the cross bridge ATPase. The ATP usage is unimolecular.

The measurable binding of Ca²⁺ to the myofibrils is unimolecular, not cooperative (Figure 2).

On cardiac cTnC there are two high affinity and two low affinity Ca²⁺ binding sites but only a total of three bind, i.e. only one for the weaker, activating sites. The strong binding sites are fully occupied under all physiological conditions. Mg²⁺ binding to any cTnC site is too weak to have the effect seen later under Magnesium inhibition. The conclusion is under physiological conditions the measurable binding of Ca²⁺ to the myofibrils is unimolecular, not cooperative.

This is fully confirmed by Morimoto and Ohsuki [3]. Only one of the low active sites is used on activation.

The competitive inhibition of Ca²⁺ activation by Mg²⁺ (Table 1).

The conclusion is that activation is by Ca²⁺ bound to cTnC cooperatively with Ca²⁺ bound to the ATP on the myosin light chain in equilibrium with inactive myosin bound MgATP. Mg²⁺ does not competitively bind to cTnC. This ensures that the initial binding of MgATP in diastole gives the completely relaxed myofibril maintaining the status quo of the stress-growth equilibrium. Ca²⁺ is replaced by Mg²⁺ on cleavage of the pyrophosphate bond in ATP. MgATP bound to myosin light chain is derived from creatine kinase rephosphorylation of the myosin bound MgADP product of the cross-bridge ATPase. Creatine kinase is piggy-back bound to cardiac myosin binding protein-C (cMyBP-C) on the thick filament.

cMyBP-C bound to Actin-Myosin is required to maintain Ca²⁺ cooperativity (Figure 3).

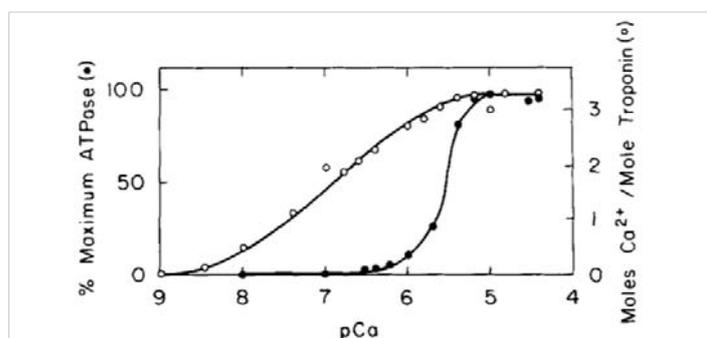(a) The reversible extraction of cMyBP-C.

Figure 2: Comparison of Ca²⁺ activation of the cardiac myofibrillar ATPase and Ca²⁺ binding to cardiac (cTnC) in the presence of 4 mM Mg²⁺. (○) and the dependence of the myofibrillar ATPase activity on free (Ca²⁺) (●), from Holroyde, [1].

Table 1: Variation with pMg of the pCa₅₀ (half activation) and Hill coefficient for Ca²⁺ activation of isometric force development in skinned cardiac-muscle fibres, from Smith, et al. [4] and original data from Donaldson [5].

pMg	4.3	3.0	2.3	2.0
pCa (half activation)	5.56	5.06	4.59	4.37
Hill coefficient (*n*)	2.92	1.89	1.86	1.62

The displacement is to the left, lower [Ca²⁺] approaching the K_m for cTnC Ca²⁺ binding (Table 2). The Ca²⁺ cooperativity is lost, lower Hill coeficient (n_H), CaATP bound is not required. This demonstrates that MgATP can act as substrate when cMyBP-C is not bound to myosin-actin.

The process of removal is reversible (Figure 4).

The conclusion is that cMyBP-C (along with cTnI see later) has the biochemical function of ensuring myosin bound MgATP is not the functional substrate of the cross-bridge ATPase in the normal heart. This ensures that the system is fully relaxed in diastole on completion of the cycle with binding of MgATP. cMyBP-C clearly has little structural function from the reversibility of its removal, save for its localisation of the creatine kinase.

(b) The addition of an N-terminal fragment of cMyBP-C that binds the myosin S2 site and actin.
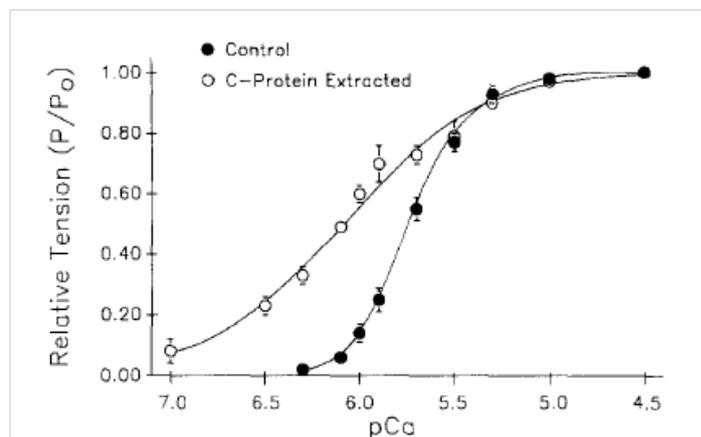
Figure 3: Tension-pCa data obtained before and after partial extraction of cMyBP-C (C-protein) from rat ventricular myocytes, from Hofmann, et al. [6], see cMyBP-C-nul later.

Table 2: The increase in pCa₅₀ and loss of cooperativity on removal of cMyBP-C.

	pCa₅₀	n_1 (P/P₀ > 0.50)
control	5.77±0.03	2.22±0.19
cMyBP-C extracted	6.13±0.03	1.20±0.16

Figure 4: Tension-pCa relationships demonstrating the effects of rat cardiac C-protein readdition to a previously extracted cardiac myocyte, from Hofmann. et al. [6].

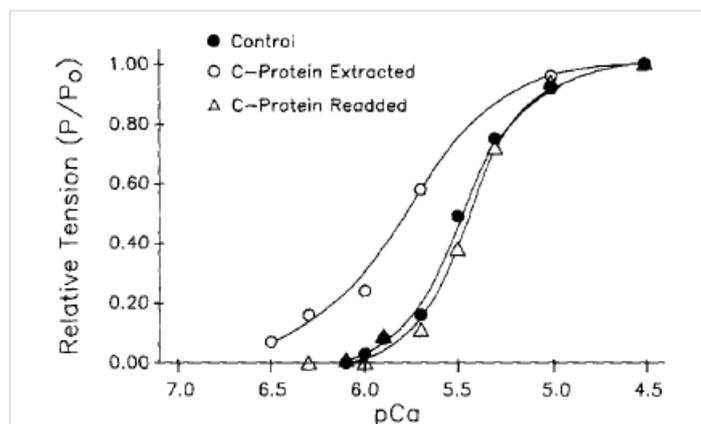Kampourakis, et al. [7] demonstrated the result of occupation of the free cMyBP-C binding sites (S2) on myosin by cMyBP-C myosin binding fragment C1mC2, see later. The free S2 binding sites outnumber those bound by a factor of at least 5. This N-terminal fragment of cMyBP-C contains the binding sites for myosin and actin binding. On addition this fragment binds to the free myosin and actin sites over-riding the effect of bound cMyBP-C [7]. At the concentration of C1mC2 used (2 μM) it almost certainly does not displace the bound cMyBP-C, as its K_m for maximal Ca^{2+} free activation is more than 20 μM. This ability to fully activate in the absence of Ca^{2+} is the first indication that the action of cMyBP-C also involves the cTnI, i.e. is concerted (Figure 5).

The displacement is to the left, lower [Ca^{2+}], and the cooperativity is lost. Only Ca^{2+} bound to cTnC is required for activation, therefore MgATP can act as substrate when C1mC2 is bound to myosin-actin. cMyBP-C bound to Myosin and/or the presence of free Myosin S2 sites are required to maintain Ca^{2+} cooperativity.

The stress/strain relationship to cardiomyopathies

I have previously suggested [8] that any mutation giving rise to the above loss of Ca^{2+} cooperativity would mean that true diastole would not be reached and the resulting chronic strain would be transmitted to the sensor in titin, releasing LIM protein and hence promoting growth. The result of increased growth is hypertrophic cardiomyopathy (HCM) [8]. Any reduction in transmission of tension to the titin would result in not maintaining the status quo of growth v. tissue loss, less Lim activity reduced maintenance growth and dilated cardiomyopathy (DCM). When mediated through change in the cMyBP-C resulting in HCM the shift in sensitivity is to the left (lower Ca^{2+}) and the opposite for DCM.

Sarcomeric mutations giving rise to a reduction in the Ca^{2+} cooperativity of activation giving rise to HCM.

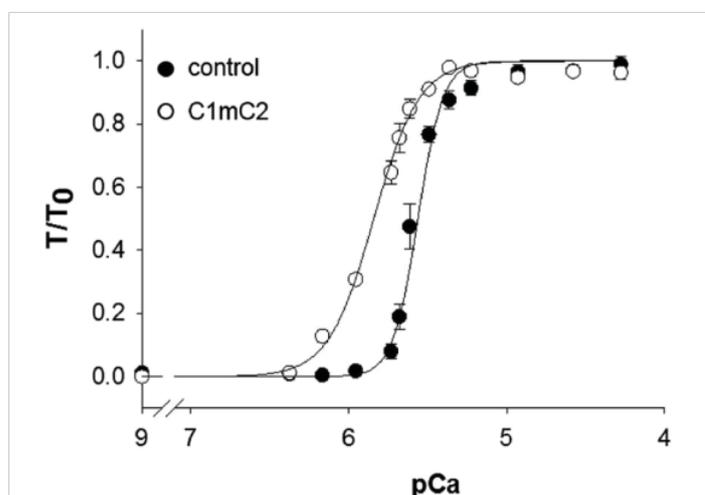Disruption of either the cTnI or the cMyBP-C functions results in the myosin bound MgATP being used as substrate, with loss of calcium cooperativity and a shift to increased Ca^{2+} sensitivity. In general the result of this is incomplete relaxation at the end of the cross-bridge cycle when MgATP is rebound and immediately used when some cTnC at diastolic calcium level ([Ca^{2+}]$_D$) is still Ca^{2+} bound, causing chronic tension in the myofibril. This tension is transmitted through the troponin-actin-titin system, with release of growth activators (LIM protein) and resulting hypertrophic growth of the myocytes.

HCM has been recently reviewed by Teekakirikul, [9]. Along with DCM it is one of the most common heritable cardiovascular disorders. HCM is characterized by left ventricular hypertrophy (LVH) that is unexplained by abnormal loading conditions, with myocyte hypertrophy and disarray, and increased myocardial fibrosis as key histopathological hallmarks. Genetic studies reveal the multiple variants in sarcomere protein genes in approximately 40% – 60% of patients with HCM, establishing HCM as a disease of sarcomere proteins, similarly for DCM. Most HCM disease-causing variants occur in the myosin, cMyBP-C and cTnI. HCM variants do also occur, along with other myopathy causing mutations, in the thin filament proteins.

I now give examples of instances of HCM where the kinetics have been graphically reported. Most of these show a small consistent loss of Ca^{2+} cooperativity reflected in reduced Hill coefficient (n_H) for Ca^{2+} activation, accompanied by increased Ca^{2+} sensitivity.

Mutations in cMyBP-C giving HCM (Figure 6).

Along with Myosin mutations these are the main sources of HCM and here the effect of cMyBP-C knockout is included.

(a) cMyBP-C mutant carriers c.2373dupG and 2864_2865DelCT.

The mutant cMyBP-C showed a shift to lower [Ca^{2+}] and reduced cooperativity. Expression of cMyBP-C was also reduced by 30% (Figure 7).

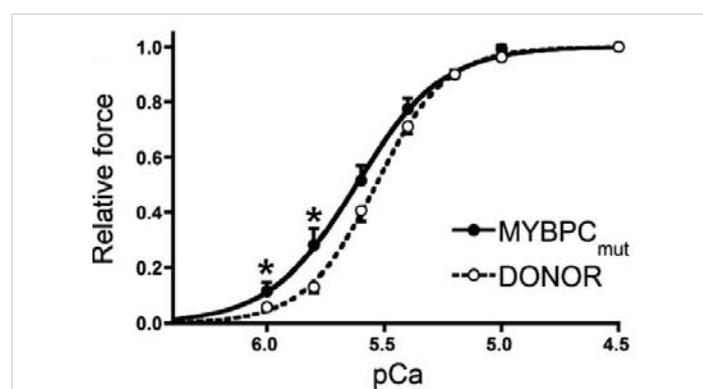Here they have also shown that for this mutation of cMyBP-C

Figure 5: The effect of binding of cMyBP-C fragment C1mC2 to acto-myosin, from Kampourakis, et al. [7].

Figure 6: Tension pCa relationship for frameshift mutants of cMyBP-C, from van Dijk, et al. [10].

there is almost complete absence of cTnI phosphorylation compared to normal. This appears to be accompanied with a decrease in phosphoryl cMyBP-C, more than the protein level decrease, and an increase in that of desmin [10]. This is the second strong link in the activities of cMyBP-C and cTnI.

(b) KO^Y235S mutation of cMyBP-C (Figure 8).

The KO^Y235S myocardium shows a left-ward shift in the pCa curve, indicating increased calcium sensitivity and reduced Ca^{2+} cooperativity. Unlike the frameshift mutants above there is no accompanying reduction in cMyBP-C expression or phosphorylation of it or the cTnI.

Parbhudayal, et al. [12] have also made the first study to demonstrate an intercellular variation of myofilament cMyBP-C protein expression within the myocardium from HCM patients with heterozygous cMyBP-C mutations.
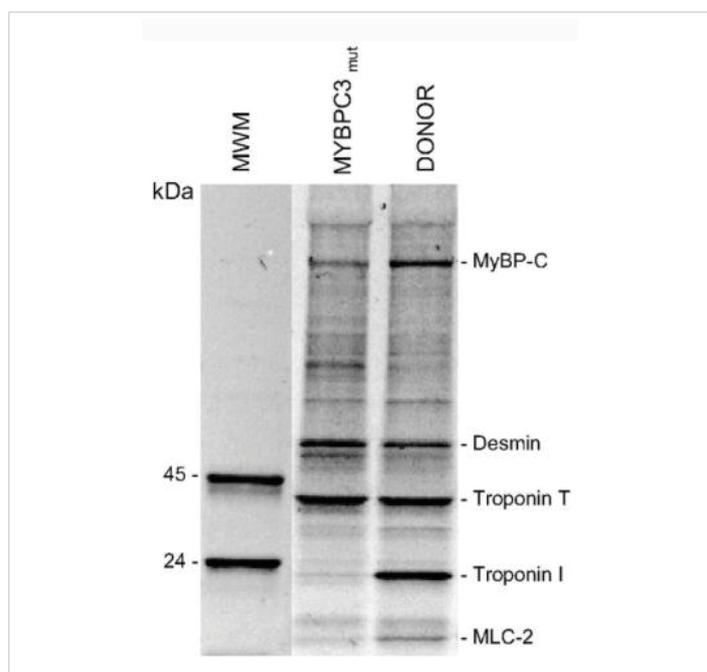
(c) cMyBP-C knockout mice.

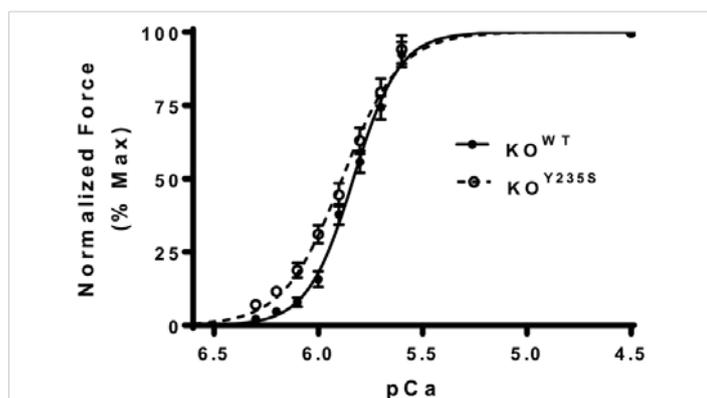**Figure 7:** The levels of phosphorylated proteins. van Dijk, et al. [10].

Figure 8: Force-pCa relationship between KO^WT and KO^Y235S of cMyBP-C, from Doh, et al. [11].

Some of the observations on the genetic elimination of the cMyBP-C are contrary to those on the extraction of cMyBP-C [6] or binding of C1C2 [7] fragment. These studies used gene targeting to produce knockout mice that lack cMyBP-C in heart. The results show that cMyBP-C is not essential for sarcomere assembly or cardiac development but that the absence of cMyBP-C is sufficient to trigger profound cardiac hypertrophy and depressed myocyte contractile properties. Clearly in the absence of the substrate control by cMyBP-C one would expect both an increase in Ca^{2+} sensitivity and loss of cooperativity. However, the finding that Ca^{2+} sensitivity of tension was reduced in cMyBP-C-nul mice contrasts with enhanced Ca^{2+} sensitivity reported after extraction of ~60% of cMyBP-C from rat myocytes using biochemical techniques. Potential explanation to account for the different results have been suggested. These are that the biochemical extractions occurred *in vitro* over short time periods, thus precluding adaptive responses, whereas compensatory effects (eg, protein phosphorylations or structural changes during hypertrophy) after genetic elimination of cMyBP-C may be additional factors in the present study. However a better explanation is in binding of the cMyBP-C to the actin-myosin, see effect of N-terminal fragments later.

The first study of cMyBP-C-nul By Harris, et al (Figure 9).

Here the anomalies found were a less than expected reduction in the Ca^{2+} cooperativity and much less than expected Ca^{2+} sensitivity, cf ref [6,7].

In a follow up Harris, et al. found [14] a more expected effect of the cMyBP-C knockout on the Ca^{2+} cooperativity, cf Hofmann [6]. But retained the lower sensitivity. In this study they surprisingly looked at the effects of adding binding fragments a la Kampourakis, et al. [7] (Figures 10-12).

Of note is the change of pCa$_{50}$ with lack of reduction in Hill coefficient on addition of C1C2 fragment to the wild-type in this later study [14] in contrast to that of others, Kampourakis, [7]. Figure 5 and Razumova [15], Figure 13. The shift to greater
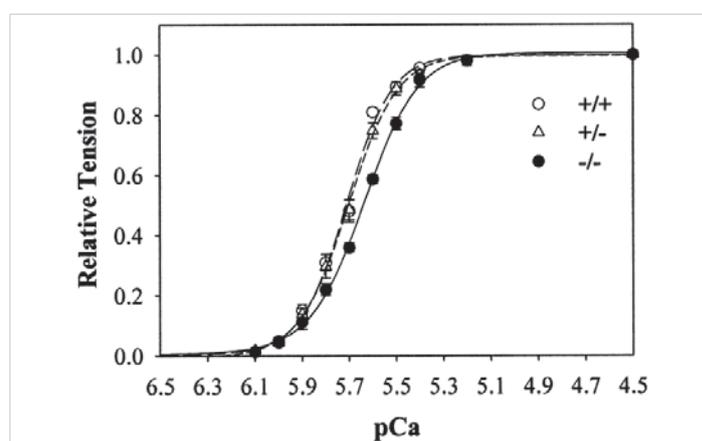

Figure 9: Ca^{2+} sensitivity of tension in skinned myocytes from cMyBP-C-nul mice. wild-type (+/+), heterozygous (+/−), and homozygous (−/−) knockout, from Harris, et al. [13].
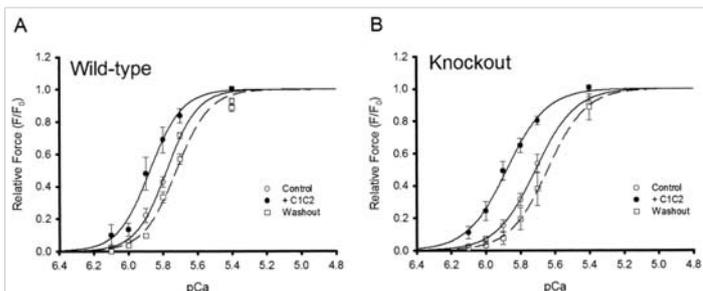
Figure 10: Effect of C1C2 peptide (10 μM) on tension-pCa relationships in permeabilized myocytes, Wild type and cMyBP-C-nul knockout, from Harris, et al. [14].
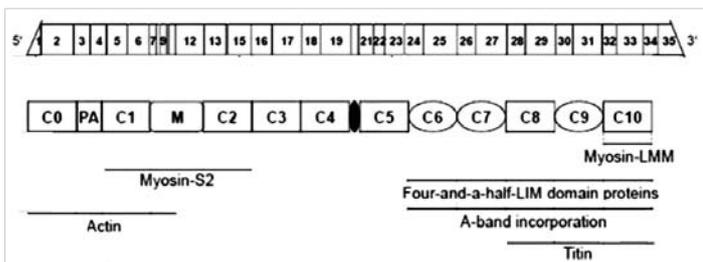

Figure 11: Schematic representation of cMyBP-C mRNA and protein structure and its interaction partners from Carrier, et al. [16].
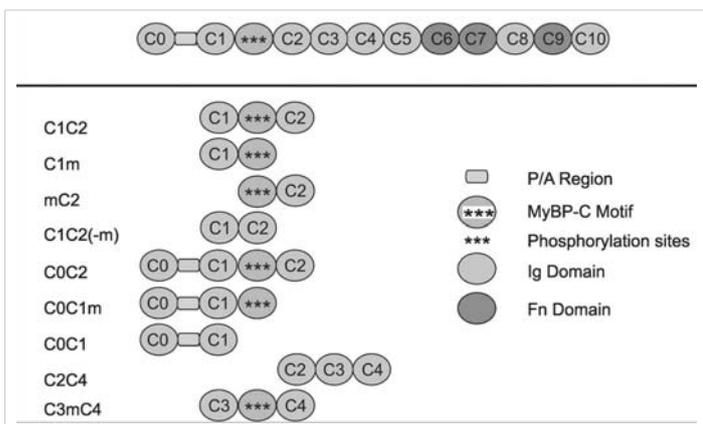

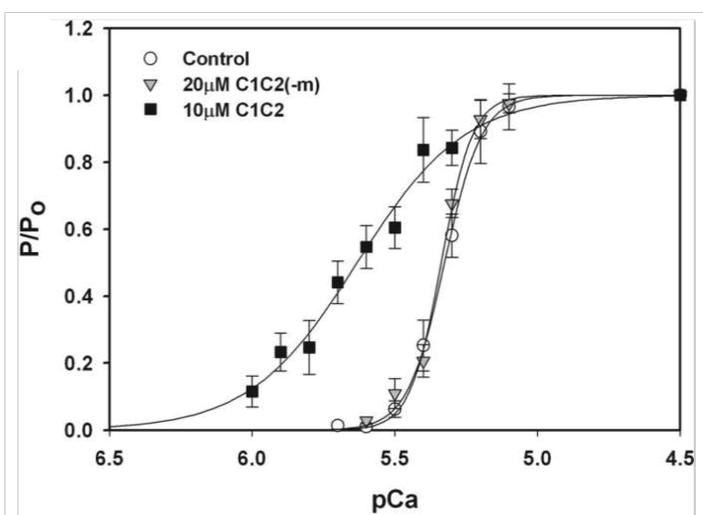**Figure 12:** Modular Domain Organisation of Cardiac cMyBP-C and the fragments synthesised [15].

Figure 13: Effects of the cMyBP-C motif on Ca²⁺ sensitivity of tension, from Razumova, [15].

Ca²⁺ sensitivity on addition of C1C2 in the absence of cMyBP-C confirms the effects of C1C2 fragment are by direct binding to unoccupied sites on the myosin (S2) and/or the actin, as suspected from the concentration used by Kampourakis. et al. [7] and the concentration they required for Ca²⁺ free activation. The dose dependency of C1C2 compared to that of C0C2 or C0C1m on the cMyBP-C KO would be interesting in this regard. The lack of shift to lower Ca²⁺ by knockout indicates a weaker affinity of the cTnC for Ca²⁺ in the absence of cMyBP-C, i.e. cMyBP-C binding to the actin-myosin increases the affinity of cTnC for Ca²⁺. The large leftward shift to greater Ca²⁺ sensitivity on addition of C1C2 fragment in the KO case does confirm this. Only partial removal of cMyBP-C, Hofmann, [6], leaves some cTnC with the higher affinity.

Limitations on use of cMyBP-C fragments.

For convenience I include here the mapping of the N-terminal fragments of cMyBP-C (Figures 11,12).

Incubation of trabeculae with 10 μM C1C2 resulted in a leftward shift of the tension–pCa relationship relative to control, indicating an increase in Ca²⁺ sensitivity of tension ($\Delta pCa_{50} = 0.30 \pm 0.05$). This confirms the incorrectness of the observation of Harris, et al. [14] (Table 3).

The addition of 20 μM C1C2(-m) that lacks the cMyBP-C motif was without effect on the tension–pCa relationship.

Note the use of only 5 μM of the N-terminal domain peptides. K_m for C1C2 Ca²⁺ free activation is 20 μM [7], when m is phosphorylated C1C2 becomes inactive. Kampourakis, et al. [7] only used 2 μM so minimising Ca²⁺ free stimulation. In experiments with cMyBP-C nulls Raumova, et al. [16] use 10 μM. The 20 μM K_m for maximum activation without Ca²⁺ probably reflects displacement of bound cMyBP-C, 2 μM only binds at free Myosin S2 and or actin sites. Hence the large reduction of Hill-coeficient on addition.

The much later observations of Harris, et al. [17] have been ignored as.

They used 100 μM of the fragments and in doing so even C1-m was found to act alone.

Mutation in myosin light chain resulting in HCM (Figure 14).

The shift to lower [Ca²⁺] and loss of cooperativity was accompanied by considerable loss of contraction strength, however this does bypass the cMyBP-C substrate control and the linkage is not floppy as with DCM mutations in light chain, see later.

Mutation in cTnI resulting in HCM (Figure 15).

The observed results of this mutation were;

1) the complex containing HCTnIR145G only inhibited

Table 3: The effects of N-terminal proteins on Ca^{2+} activation [16].

| Protein (n)	[ ] (µM)	pCa_{50}	pCa_{50}	ΔpCa_{50}	Hill coef. (nH)	Hill coef. (nH)
		control	+ protein		control	+ protein
C0C2(6)	5	5.32±0.02	5.73±0.04	0.42±0.04	5.43±0.32	2.19±0.14
C1C2(4)	5	5.30±0.02	5.54±0.05	0.25±0.05	6.45±0.86	2.65±0.25
C0C1m(4)	5	5.32±0.01	5.59±0.05	0.27±0.05	7.66±0.72	2.71±0.34
C1m(6)	5	5.34±0.02	5.46±0.04	0.12±0.04	6.83±0.89	3.72±0.34
C1C2(5)	10	5.29±0.02	5.58±0.05	0.30±0.05	5.02±0.33	2.11±0.14
mC2(4)	10	5.30±0.01	5.32±0.03	0.02±0.03	6.63±0.44	5.90±0.67
C0C1(3)	10	5.34±0.01	5.32±0.01	-0.02±0.01	6.61±0.70	7.50±0.83
C2C4(4)	20	5.32±0.01	5.32±0.01	0.00±0.01	6.73±0.21	6.15±0.71
C1C2m(3)	20	5.33±0.02	5.34±0.01	0.01±0.01	9.62±2.08	8.67±2.04
C3mC4(3)	20	5.30±0.02	5.29±0.01	-0.01±0.01	4.96±0.62	5.56±0.42
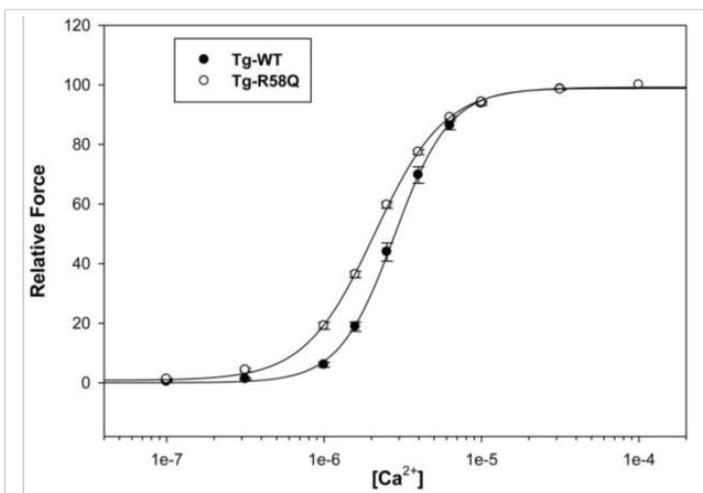
Figure 14: The effect on Ca^{2+} sensitivity of force determined in Tg-R58Q glycerinated skinned papillary muscle fibers compared to Tg-WT, from Mettikolla, et al. [18].
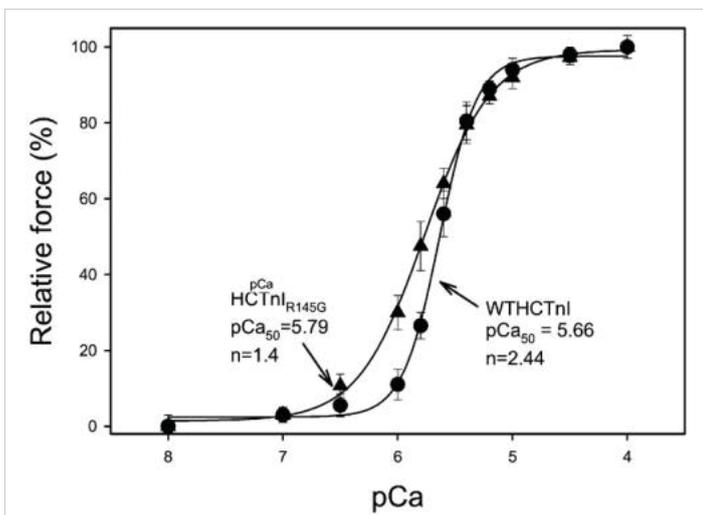

Figure 15: Activation of actin-Tm-activated myosin ATPase activity by either WTHCTnI or HCTnI$_{R145G}$, dependence on Ca^{2+}, from Lang, et al. [19].

84% of Ca^{2+}-unregulated force. Incomplete relaxation at diastolic Ca^{2+}, chronic stress.

2) the recovery of Ca^{2+}-activated force by HCTnIR145G was decreased and

3) there was a significant increase in the Ca^{2+} sensitivity of force development.

Restrictive cardiomyopathy (RCM) with cTnI mutations.

There are reports of restrictive cardiomyopathy (RCM) showing major loss of Ca^{2+} cooperativity arising from mutations mostly in cTnI. RCM is a more pronounced form of HCM. Two examples are given here, both from Davis, et al. (Figures 16,17).

PKA treatment shifts both curves to the right, less Ca^{2+} sensitive without change in Hill coefficients.

RCM is when the walls of the lower chambers of the ventricles are too rigid to expand as they fill with blood. The pumping ability of the ventricles may be normal, but it's harder for the ventricles to get enough blood. With time, the heart can't pump properly. This leads to heart failure. With RCM mutants the loss of cooperativity and shift to greater Ca^{2+} sensitivity are the largest of those reported for HCM and more reflect those found when cMyBP-C is nullified by addition of its S2 binding moiety or partially removed. There is considerable activation and lack of relaxation at low $[Ca^{2+}]_D$ [20,21]. There is no evidence of amyloidosis giving rigidity in this case, as shown by Du, et al. [22], however increases in desmin and α-actinin have been demonstrated in diastolic failure by Zhang, [23] and Sheng, et al. [24]. The latter may very well be the basis of the increase in rigidity found in RCM.

Other RCM related cTnI changes are reported by Parvatiyar, et al. [25], Jean-Charles, et al. [26], Wen, et al. [27] and Gomes, [28].

With the above large shift to increased Ca^{2+} sensitivity the suggestion is that cTnI is involved with the binding of cMyBP-C to the actin-myosin, the third strong indication of this.

A mutation cTnC giving unexpectedly DCM although loss of Ca^{2+} cooperativity.

There is one instance of mutated cTnC where the use of MgATP as substrate will occur and should lead to hypertrophy but this is over-ridden by breakdown in stress transfer to the thin filament causing dilated myopathy (Figure 18).

I conclude here that the Ca^{2+} sensitivity increase is entirely down to a structural change i.e. cTnI not being G159C-cTnC
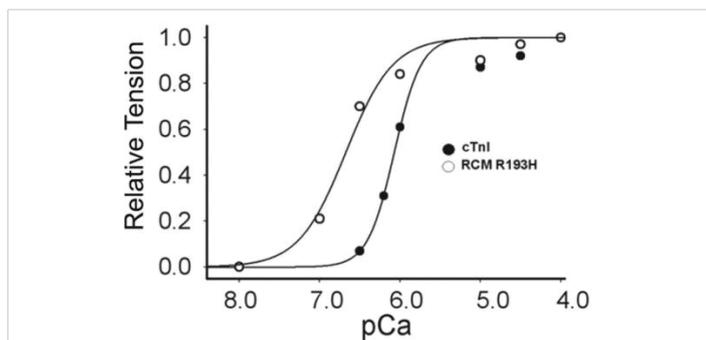
Figure 16: Ca²⁺-activated tension in single permeabilized R193H cTnI mutant cardiac myocytes, from Davis, et al. [20].
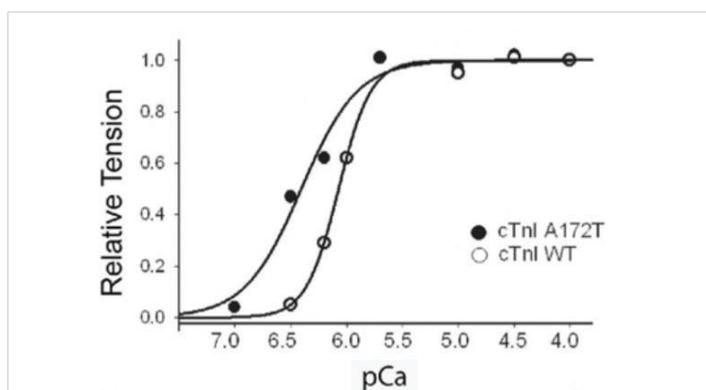

Figure 17: Ca²⁺-activated tension in single permeabilized A172T cTnI mutant cardiac myocytes, from Davis, et al. [21].
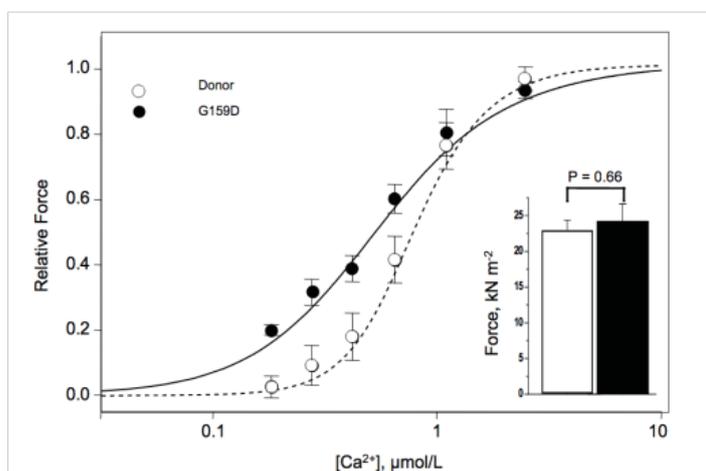

Figure 18: Contractile properties of skinned donor and G159D cTnC myocytes Inset, Maximum force in skinned myocytes from human donor (open bar) and G159C cTnC tissue (filled bar), from Dyer, et al. [29].

bound [27] and thus not cooperating with the cMyBP-C in blocking the use of MgATP as substrate. It is also likely that G159C-cTnC does not properly bind other thin filament proteins, actin, cTnT or tropomyosin. Similar results were found by Swindle, et al. [30].

Sarcomeric mutations that do not show loss of Hill coefficient, Ca²⁺ cooperativity, giving rise to either HCM or DCM

These mutations are mostly in the Troponin-T-tropomyosin-titin genes although those in myosin are

reported. The mutations either increase or decrease the transfer of stress to the titin and its bound Lim protein, giving rise to HCM or DCM. The Ca²⁺ cooperativity is maintained, i.e. both the cTnC and myosin bound ATP are Ca²⁺ bound with these mutations. The central roles of Troponin-T (cTnT) and tropomyosin (∝-Tm) in the thin filament transfer of stress is well demonstrated by the presence of assorted variants with either hypertrophic or dilated outcome. The HCM mutants of ∝-tropomyosin (∝-Tm) all display elevated Ca²⁺ free ATPase (or tension), a form of inhibited diastolic relaxation, different to loss of Ca²⁺ cooperativity, again giving chronic stress in the titin. In general the various mutants giving DCM show reduced maximum ATPase/tension, little or no shift in either the pCa_{50} or Hill coefficient.

Mutations in the Myosin Heavy Chain

For the myosin heavy chain variants there are very little data on the variation of ATPase or contractility with [Ca²⁺] although there are a very large number of mutations known. All available are HCM and show increased maximum contractility at high [Ca²⁺]. The earliest found was Keller, et al. [31] who showed that mutant myosin R403W exhibited a large increase in maximal actin-activated ATPase activity (+114%; $p < 0.05$) and Km for actin (+87%; $p < 0.05$) when compared to WT. Kraft, et al. [32] found increase in contractile strength for R723G in cardiomyocytes. Spudich, et al. [33] studied the mutations R21C, S166F (small n_H increase) and DK177 all showed increase maximal ATPase. Nag, et al. [34] for HCM R403Q Show a lowering of contractile strength. It lacks the S2 fragment (cMyBP-C binding domain) and thus must surely behave like cMyBP-C-nul. Sarkar, et al. [35] confirm the R403Q conclusions. These all increase tension transfer to titin.

Mutation in myosin light chain giving DCM (Figure 19)

This light-chain mutation was seen to impair the binding of the regulatory light chain (RLC) to the myosin heavy chain (MHC). Less binding to MHC, more floppy less strain is transferred to the thin filament.

Tropomyosin (∝-Tm) mutations giving HCM

Michele, et al. [37] report the functional effects of cardiac-specific expression of human E180G mutant ∝-Tm in transgenic mice (Figure 20).

E180G ∝-Tm mice had significantly slowed relaxation under physiological conditions. This dysfunction was eliminated by propranolol. In a follow up by Michele, et al. [38] they use adenoviral-mediated gene transfer of four point mutations in α-tropomyosin (∝-Tm) into adult cardiac myocytes in vitro to show that all four HCM α-Tm proteins can be expressed and incorporated into normal sarcomeric structures in cardiac myocytes at similar levels as normal α-Tm proteins.

Muthhuchamy, [39] report the functional effects of cardiac-specific expression of HCM human α-TM 175 TG in transgenic mice (Figure 21).
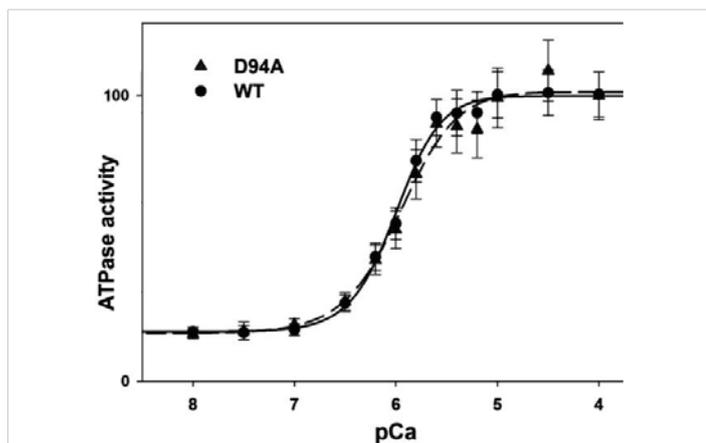
Figure 19: The ATPase-pCa relationship in WT and D94A reconstituted porcine cardiac myofibrils, from Huang, et al. [36].
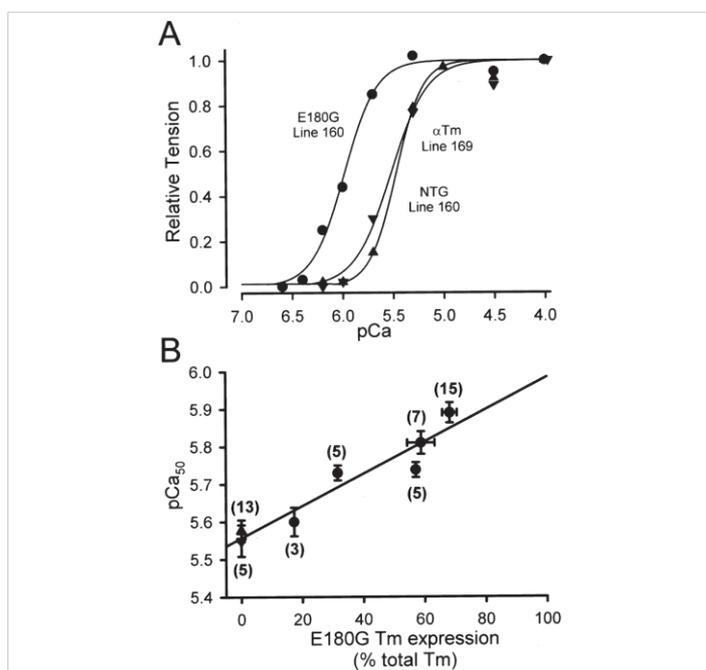

Figure 20: E180G ∝-Tm transgene dose dependence of increased Ca²⁺ sensitivity of force production in single cardiac myocytes. A, Ca²⁺-activated force generation in representative single cardiac myocytes isolated from NTG (▲), 169 WT ∝-Tm (▼), and 160 E180G ∝-Tm (●) mice. B, E180G ∝-Tm transgene dose dependence of increased Ca²⁺ sensitivity of force production, from Michele, et al. [37].
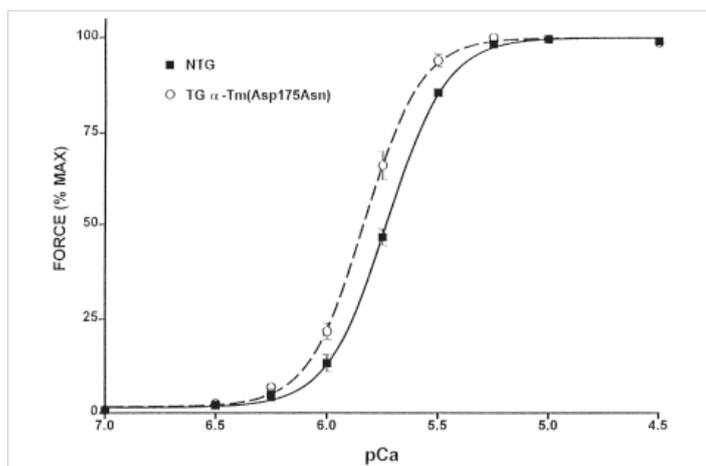

Figure 21: Normalized pCa-force relation of skinned fibre bundle preparations from NTG and α-TM 175 TG (line 108) mouse hearts at sarcomere length 2.0 μm.

Increased expression of the α-TM 175 transgene in different lines causes a concomitant decrease in levels of endogenous α-TM mRNA and protein expression. In vivo physiological analyses show a severe impairment of relaxation in hearts of the HCM mice. In this case the stress was elevated in diastole. Chang, et al. [40] report similar results with other HCM-associated ∝-Tm mutations (Figure 22).

All three HCM-associated ∝-Tm mutations increased the Ca^{2+} sensitivity of ATPase activity. All three had significantly decreased abilities to inhibit ATPase activity at effectively zero $[Ca^{2+}]$. This is clearly the same as with the α-TM 175 transgenic above [39]. In all these the $[Ca^{2+}]_D$ chronic stress is transferred to the titin.

∝-Tm mutants giving DCM (Figure 23).

Statistical analysis of activities at pCa 9.1 and 5.0 shows no significant differences; Both mutations caused a small but significant decrease in the pCa_{50} values, and had no effect on the inhibition of ATPase activity. from Chang, et al. [40]. Somehow less tension in titin, need to look for altered interaction with cTnT etc.
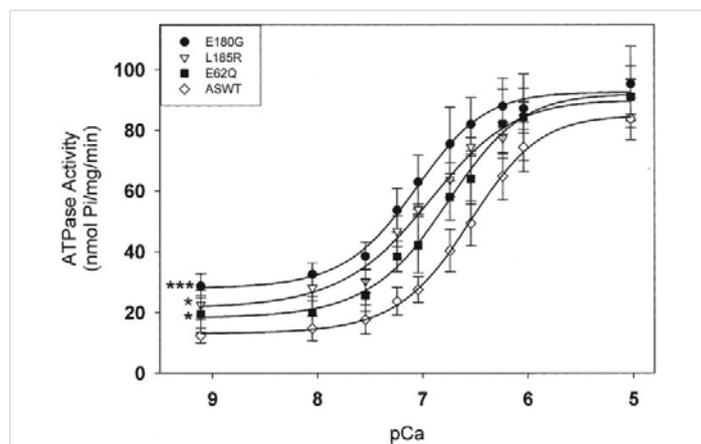

Figure 22: Effects of HCM-causing ∝-Tm mutations on the Ca²⁺-dependent myofibrillar ATPase activity rates. Chang, et al. [40].

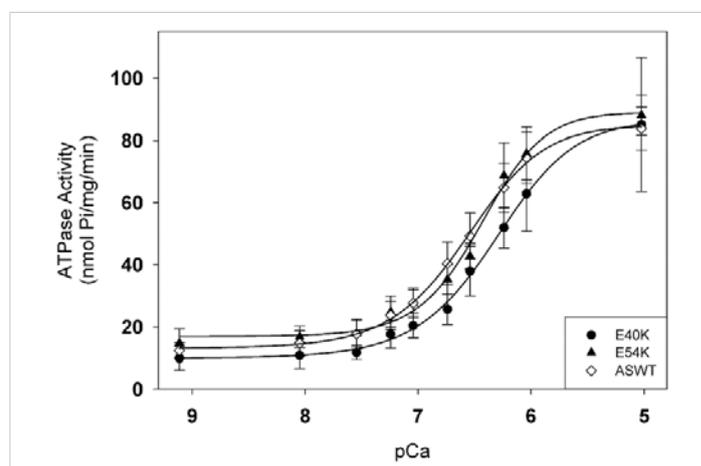**Figure 23:** Effects of DCM-causing ∝-Tm mutations, E40K and E54K, on Ca²⁺-dependent myofibrillar activity rates compared to WT.

Various reports of other HCM and DCM Tm mutants

Gupte, et al. [41] showed DCM mutants D84N and D230N shift the pCa_{50} stimulated ATPase activity to the right relative to WT and HCM mutants shift the pCa_{50} to the left. The changes in pCa, n_H and $ATPase_{max}$ for the DCM cases are small. The changes in the curves for the HCM mutants are again similar to above ∝-Tm reports and there is residual activity at diastolic $[Ca^{2+}]_D$. Similar DCM results are reported by Rajan, et al. [42] for a-TM54 TG hearts with more significant loss in contraction force (Table 4) (Figure 24).

Actin mutant giving DCM

There is no cooperativity change and a small movement to lower $[Ca^{2+}]$. There was no significant difference in sliding speed or fraction of filaments motile. When troponin was dephosphorylated the Ca^{2+}-sensitivity of E361G-containing thin filaments was now much lower than NTG, this was due to uncoupling of Ca^{2+}-sensitivity from cTnI phosphorylation.

cTnT mutations giving HCM

Hernandez, et al. [44] report both ATPase and force measurements for skinned papillary muscle fibers of F110I-cTnT and R278C-TnT transgenic mice. In both F110I-TnT and R278C-TnT the maximum force was greatly reduced from WT but the ATPase not affected. In this case it is clear that the force instead of transmitting to the Z disc was absorbed by the titin spring, and thus releasing increased LIM protein.

cTnT mutations giving both HCM and DCM

(a) human wild-type, ΔK210 (DCM), or ΔE160 (HCM) cTnT (Figures 25,26).

Table 4: Ca²⁺-tension in skinned fibre bundles of a-TM54 TG hearts [42], DCM.

Group	pCa_{50}	n_H	Force (mN/mm²)
NTG (5 months old)	5,75±0.02	3.38±0.40	37.50±4.04
Moderate copy TG	5.67±0.01	3.77±0.21	25.41±1.57
NTG (1 month old)	5.93±0.03	3,74±0.23	46.09±7.22
High copy TG, 1.9	5.82±0.03	3.78±0.15	22.35±1.48
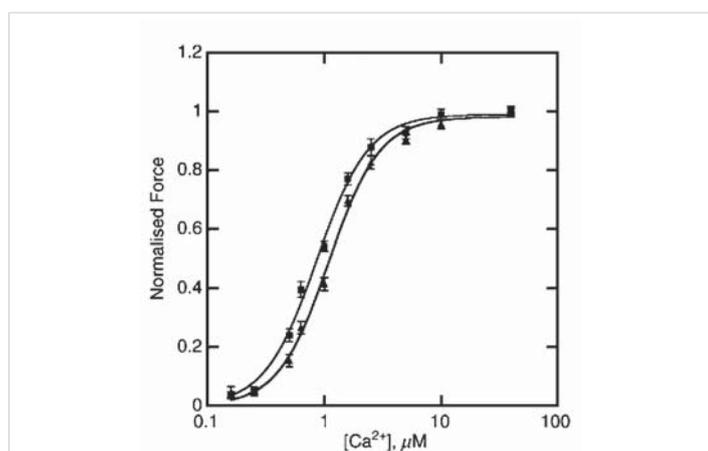
Figure 24: The dependence of isometric force on [Ca²⁺] for 8 NTG(▲) or ACTC E361G mice (■), from Song, et al. [43].

Venkatraman, et al. [47] report similar results for other DCM mutants.

(b) HCM cTnT-I79N mutation (I79N). DCM knock-in mouse model carrying the heterozygous TnT-R141W mutation (HET) (Figure 27).

It appears that the transfer of stress to the titin is opposite in the two cases and when both are included together they nullify each other. In this case there are small changes in Hill coefficient, origin of which is not clear.

Sommese, et al. [49] report on mutants used above (Table 5).

Total disagreement with Cooperativity of WT reported by Holroyde [1] and Smith [2] and very little Hill coefficient (n_H) change with mutations. This is expected as only the subfragment S1 is used. It is S2 that binds the cMyBP-C. HCM, DCM mechanisms unclear.

DCM From several patients no protein mutation known

In samples from patients Wolff, et al. [50] report that the calcium concentration producing half-maximal tension
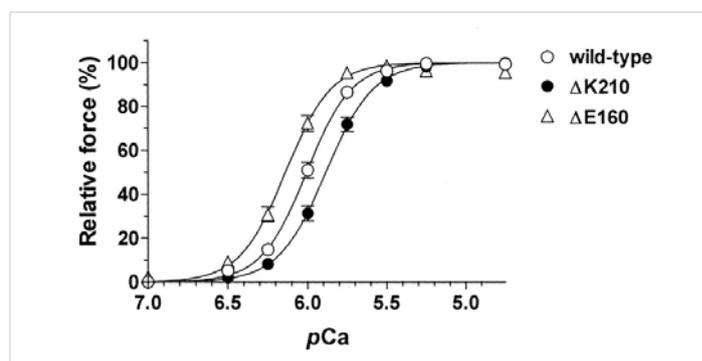
Figure 25: Force–pCa relationships in the rabbit skinned cardiac muscle fibers into which human wild-type, ΔK210 (DCM), or ΔE160 (HCM) cTnT was incorporated. Maximum force levels were both ca. 30% down compared with wild-type, from Morimoto, et al. [45].
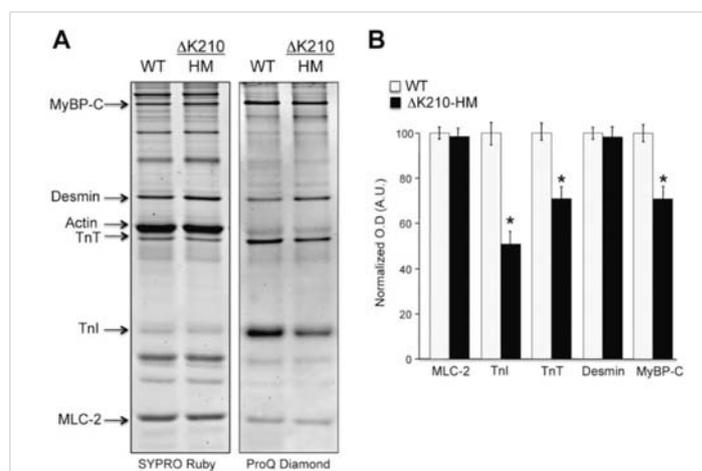
Figure 26: Differential phosphorylation of sarcomeric proteins in isolated myofibrils from ΔK210 homozygous hearts (DCM). A, cardiac myofibrillar proteins stained with ProQ Diamond for phospho-proteins detection and Sypro Ruby for total protein detection. They show equal protein content (Sypro) and disparity in phosphorylation levels (ProQ), from Sfichi-Duke, et al. [46].
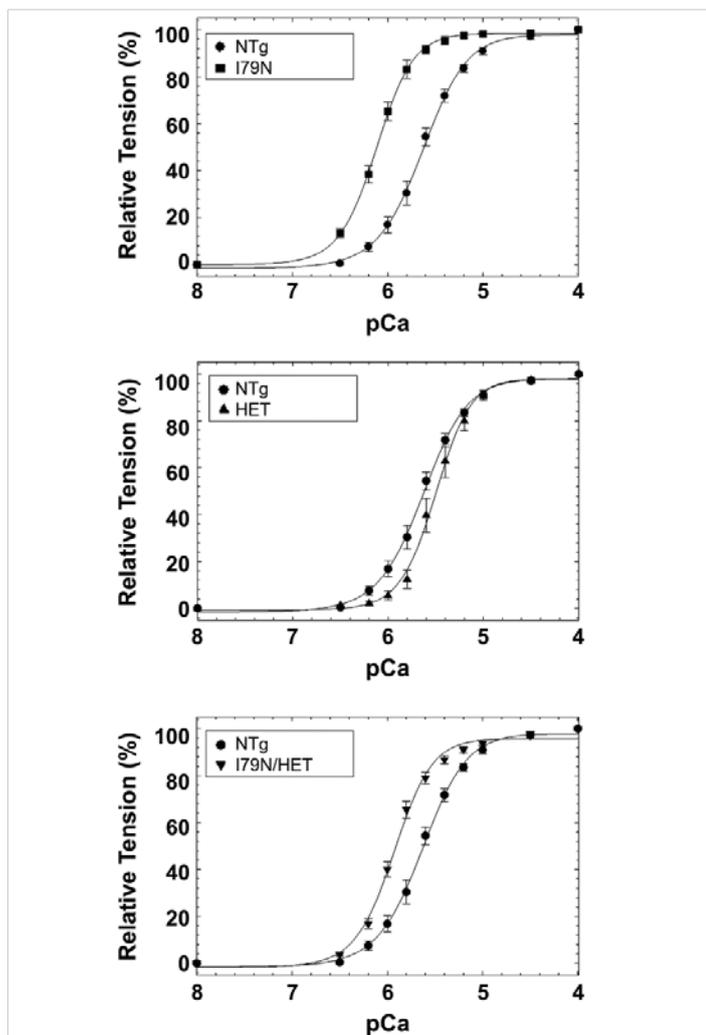
Figure 27: Calcium-dependent tension of, A, I79N mice compared to NTg. B, R141W HET mice compared to NTg. **C,** I79N/HET mice mutually normalized the Ca²⁺ sensitivity towards WT, from Jones, et al. [48].

Table 5: ATPase activity of cardiac S1 with assorted HCM and DCM TnT mutants [49].

TnT	disease	pCa$_{50}$	ΔpCa$_{50}$	% max.activity	% min.activity	n_H
WT		6.17±0.01	-	100±1	10±1	1.2±0.1
179N	HCM	6.44±0.04	+0.27	100±2	7±1	1.2±0.1
E163K	HCM	6.36±0.03	+0.19	147±7	15±1	1.4±0.2
R141W	DCM	5.96±0.02	-0.21	93±2	5±1	1.2±0.1
R173W	DCM	6.08±0.01	-0.09	101±4	6±1	1.5±0.1

([Ca²⁺]$_{50}$) was less in cardiomyopathic preparations than in donor preparations demonstrating an increase in myofibrillar calcium sensitivity of isometric tension which is lost on pKA phosphorylation. These results are contradictory to all other reports and probably reflect/suggest that the increased calcium sensitivity in this DCM report may be due at least in part to an in vivo reduction of the beta-adrenergically mediated phosphorylation of myofibrillar regulatory proteins such as cTnI and/or cMyBPC or their degradation.

Conclusion

The effects of HCM mutants of cTnI, in particular the large shifts in pCa$_{50}$ for activation of the RCM mutants along with the two earlier indications of a concerted effect of cMyBP-C in conjunction with cTnI lead to a definite observation. The conclusion is together in the normal heart the cMyBP-C in conjunction with cTnI have the biochemical function of ensuring myosin bound MgATP is not the functional substrate of the cross-bridge ATPase. Disruption of either cTnI or cMyBP-C is sufficient to break this and allow MgATP to be used as substrate of the cross-bridge ATPase. The result of this is clear with a reduction in the Ca²⁺ cooperativity of activation and HCM. When unimpaired the joint action ensures that the system is fully relaxed in diastole on completion of the cycle with binding of MgATP. The cMyBP-C knock out myofibril does not display the commonly accepted cTnC affinity for Ca²⁺ it shows activation at higher [Ca²⁺]. Clearly one of the functions of cMyBP-C is to increase the calcium affinity of cTnC to that we are familiar with. The interaction with actin-myosin gives a change in Ca²⁺ sensitivity when an N-terminal fragment of cMyBP-C is added to the knock out myofibril. The phosphorylation state of the cTnI or others may have a bearing on this as well as the degree of pCa$_{50}$ change with the mutants. The subtle interaction between cTnI and cMyBP-C is in need of much careful study. The overlap of the actin and myosin binding regions of cMyBP-C are certain candidates for the concerted action in substrate control. The possible binding of C1mC2 fragment of cMyBP-C to the actin along with the myosin is not clear in the literature reports. The effect of binding assorted segments of cMyBP-C in its absence may illuminate this.

The mechanisms of the effects, not arising from the cMyBP-C/cTnI system, of a range of mutations giving HCM and DCM are not clear. However when studying the response to calcium with an array of mutant sarcomeric proteins one may rationalise the effect of a mutation from the accepted function and interactions of the particular protein. Generally stronger interaction, more stress in filaments, more LIM release giving HCM and the reverse for DCM. Examples include floppy binding of MLC to the myosin giving DCM with retained ATPase function. Tropomyosin (α-TM) is involved in the interaction of the MLC with the actin filament, the crossbridge. All the HCM mutants of α-TM show a hindered dissociation of the crossbridge, slowed relaxation, resulting in transfer of chronic tension to the titin. Other α-TM mutants show reduced tension, at both or either [Ca²⁺]$_D$ and [Ca²⁺]$_{sys}$ resulting in DCM. TnT is directly involved with the stress transfer to the thin filament titin and hence mutations result in either HCM or DCM. Only one actin mutant is quoted giving weaker thin filament stress. Clues to the effect of mutations may be found in structural studies such as given by Lu, Wu and Morimoto, [51] and a review by de A. Marques M and de Oliveira [52].

I stress that all my conclusions regarding Ca²⁺ and Mg²⁺ in the myofibril are based entirely on the well respected measurement made on the intact system by Holroyde, Robertson, Johnson, Solaro and Potter, Morimoto and Ohtsuki [3], Donaldson, best and Kerrick [5], and myself et al. [2,4]

and others in these references. To emphasise the need to only consider the intact system with all the many interactions of its constituents, I quote a more recent source Harris, Rostkova, Gautel and Moss14 who clearly demonstrate that the binding of cMyBP-C to the system has a major effect on the Ca^{2+} affinity of the cTnC, This is especially shown by the effect of adding the actin-myosin binding fragment of cMyBP-C (C1mC2) to the cMyBP-C knockout myofibrils.

A paper has just appeared that confuses the issue, but it can be ignored as all the measurements were performed on the Naked protein cTnC and a fragment of it, Rayani K, Seffernick JT, Li YA, Davis JP, Spuches AM, Van Petegem F, Solaro RJ, Lindert S, and Tibbits GF. Binding of Calcium and Magnesium to Cardiac Troponin C. doi: https://doi.org/10.1101/2020.06.14.150854. This article is a preprint and has not been certified by peer review.

Acknowledgement

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