Molecular Basis for the Influence of Muscle Length on Myocardial Performance

ÁRVIND BABU, EDMUND SONNENBLICK, JAGDISH GULATI*

According to Starling's law of the heart, the force of contraction during the ejection of blood is a function of the end-diastolic volume. To seek the molecular explanation of this effect, a study was made of the effects of length on Ca^{2+} sensitivity during tension development by isolated demembranated cardiac muscle in which the cardiac form of troponin C was substituted with skeletal troponin C. The results of troponin C exchange were compared at sarcomere lengths of 1.9 and 2.4 micrometers. Enhancement of the myocardial performance at the stretched length was greatly suppressed with the skeletal troponin C compared with the cardiac troponin C. Thus the troponin C subunit of the troponin complex that regulates the activation of actin filaments has intrinsic molecular properties that influence the length-induced autoregulation of myocardial performance and may be a basis for Starling's law of the heart.

INCREASES IN DIASTOLIC VOLUME OF the heart during its operation as a pump are associated with graded improvements in cardiac performance (*1*). This finding, that the force of contraction during the ejection of blood is a function of the heart size in diastole, was made nearly 100 years ago and is known as Starling's law of the heart (*1–6*). In subsequent studies with isolated cardiac muscle, the tension that could be developed in response to stimulation was also found to increase markedly when the length of the sarcomeres was increased from 1.4 to 2.5 μm (*3, 4*). This increase in tension with length is now considered to be the physiological basis for the effect of heart size on cardiac performance (*5, 6*), but the molecular mechanisms for the effect are unknown.

We have examined the role of troponin C (TnC) in the muscle length–induced improvement of cardiac performance. TnC is the Ca^{2+} binding subunit of the troponin complex that controls the onset of muscle contraction during normal activation (*7*). The relation between pCa and force (pCa equals $-\log[Ca^{2+}]$) of the demembranated myocardium as a function of muscle length have previously indicated that Ca^{2+} sensitivity is increased at longer sarcomere lengths (*8, 9*). However, the length-induced shift of the relation between pCa and force is significantly less in skeletal muscle than in cardiac muscle (*10–13*). We now show that when native cardiac TnC (CTnC) is extracted from isolated cardiac muscle and replaced with purified TnC from skeletal muscle (STnC), the sensitivity of the muscle to length change is considerably reduced. The results suggest that, in addition to its role in muscle activation, CTnC also modulates the size-induced changes in cardiac performance in response to physiological (and pathophysiological) perturbations and may provide a molecular explanation of Starling's law.

Skinned trabeculae from the ventricles of hamster hearts were prepared by chemical treatment in the presence of Lubrol-wx (0.5% v/v) detergent (*7*). Each trabecula was selected for sarcomere uniformity and sharp laser pattern. Usually only one trabecula in a given heart was satisfactory. Skeletal TnC was exchanged for CTnC as described before (*7*) except that potassium propionate was used instead of potassium chloride.

When trabeculae were reconstituted with either CTnC or STnC and then activated with Ca^{2+} (Fig. 1), the recovery of tension was similar for the two types of TnC. Analysis of the trabeculae on SDS-polyacrylamide gels indicated 70 to 75% deletion of TnC with extraction and a corresponding loss of Ca^{2+} activation (*14, 15*). After reconstitution, the CTnC content was fully restored (Table 1). The STnC band nearly overlapped with the cardiac myosin light chain-2 (LC2) band [see also figure 4 in (*7*)], and the intensity increase in this combined band showed that STnC also adsorbed to the denuded CTnC sites.

Comparison of the pCa-force relationships at a long (2.3 to 2.4 μm) and a short (1.9 μm) sarcomere length under the conditions used in this study indicated that the length-induced shift of the activation curves was dependent on the type of TnC in the myocardium (Fig. 2). The length-induced

Table 1. Quantitation of the bands shown in lanes 1 to 3 in Fig. 1C. The band intensities in each case were normalized to the corresponding LC1 band as described earlier (*7*). Similar results were obtained in four other analyses. Note that in each case of the trabeculae reconstituted with STnC [(+)STnC] the added STnC ran with the LC2 band and this is reflected in the value of the LC2 intensity.

Fiber treatment	CTnC	LC2	LC1
Native	0.31	0.81	1.00
(+) STnC	0.09	0.96	1.00
(+) CTnC	0.33	0.84	1.00

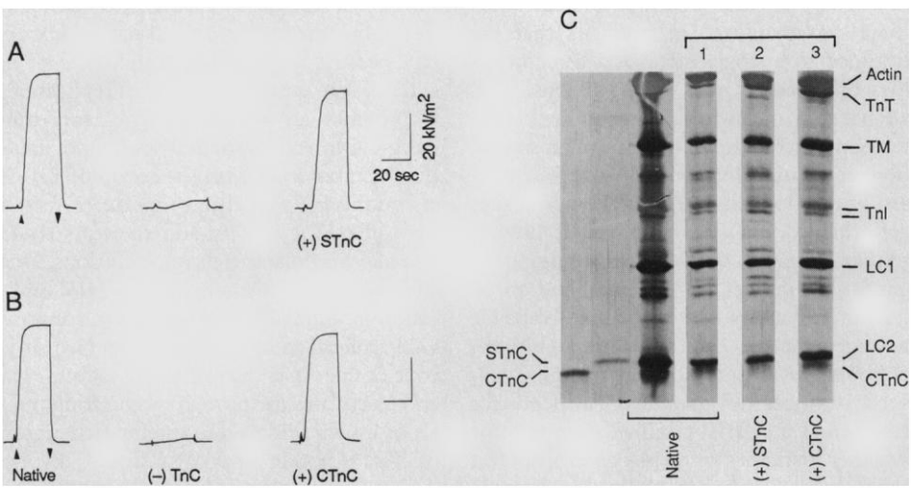

Fig. 1. (**A** and **B**) Recovery of tension with (A) STnC and (B) CTnC. The TnC was extracted at 30°C in 5 m*M* EDTA, 10 m*M* imidazole, *p*H 7.2, for 20 to 30 minutes, and the trabeculae were reconstituted with STnC or CTnC in the relaxing solution (m*M*: 80 potassium-propionate, 5 EGTA, 5 $MgCl_2$, 20 imidazole, 5 adenosine triphosphate, 20 phosphocreatine, *p*H 7.0). The force traces are in response to activation with pCa4 (20°C) [see (*7, 24*)]. The recovered force was between 85 and 100%. (**C**) Analysis of trabeculae by SDS–polyadenylated gel electrophosesis (15% polyacrylamide, silver-stained). The first two lanes are the purified STnC and CTnC to mark the displacement of the bands. The third lane is an oversized native cardiac preparation (not experimental); this overloaded lane serves to indicate the TnC band in the cardiac muscle. The next three lanes (marked 1, 2, and 3) are experimental trabeculae: native, STnC-loaded, and CTnC-loaded, respectively. TnT, troponin T; TM, tropomyosin; TnI, troponin I.

A. Babu and E. Sonnenblick, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461. J. Gulati, Departments of Medicine and Physiology/Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461.

*To whom correspondence should be addressed.

shift obtained with the STnC-loaded trabecula in Fig. 2 was reduced (by one third) compared to the CTnC-loaded trabeculae. This reduction offers a straightforward explanation for the difference in the response of skeletal and cardiac muscles, that is, the CTnC moiety itself in the sarcomeric assembly is the transducer that adjusts the Ca^{2+} sensitivity of the myofilaments as a function of length (*16, 17*).

Finding that phasic contraction in skinned cardiac cells triggered by free Ca^{2+} was sensitive to length, Fabiato and Fabiato (*4*) suggested a role for the Ca^{2+}-induced Ca^{2+} release (*18*) in the length-dependent effects on tension. Although this remains an important idea, it can be argued on the basis of differences with CTnC and STnC that Ca^{2+}-induced Ca^{2+} release is not the exclusive mechanism for the length-dependent effect on cardiac tension.

Similarly, it has often been suggested that Ca^{2+} sensitivity of cardiac muscle might be improved with increased numbers of attached bridges (*5, 6*). A major problem with this idea, as noted previously (*6*), is that Ca^{2+} sensitivity increases even at lengths into the descending limb of the length-tension relation where the number of bridges would decrease. It is possible, of course, that the mechanisms are different above and below 2.4 μm, or that several different mechanisms cooperate to produce the length-induced shifts of Ca^{2+} sensitivity in the two domains. Our findings now indicate that changes in bridge number are not the driving mechanism for the length-dependent effect on Ca^{2+} sensitivity below 2.4 μm, but rather that the presence of fewer bridges at shorter lengths is itself the consequence of a property of TnC. In Fig. 3, the steeper line for the 50% activation level of CTnC-loaded trabeculae compared to the STnC-loaded trabeculae at the same activation level, or compared to the line that would be expected from the overlap of actin and myosin filaments within the sarcomere, provides direct evidence for the following: (i) the activation process makes the main contribution to the length-tension relation of cardiac muscle under physiological conditions and (ii) the Ca^{2+} sensitivity of the particular TnC results in more or less force because the difference in the Ca^{2+}-TnC interaction modulates the number of cross-bridge attachments.

In the inset of Fig. 3 we compare the relation between length and stress of the left ventricle in anesthetized dogs (*19*) with our force data on native trabeculae for 50% and 30% activations. The relation between systolic length and stress in the beating heart is a convenient representation of Starling's law. The steeper length-force relation in the skinned muscle at lower levels of activation indicates that Starling's effect operates in the whole heart when the contractile machinery is partially activated. It is interesting that systolic pressures (100 to 150 mmHg) in the heart during normal function also correspond to activations less than 50% if one considers the appropriate geometric factors

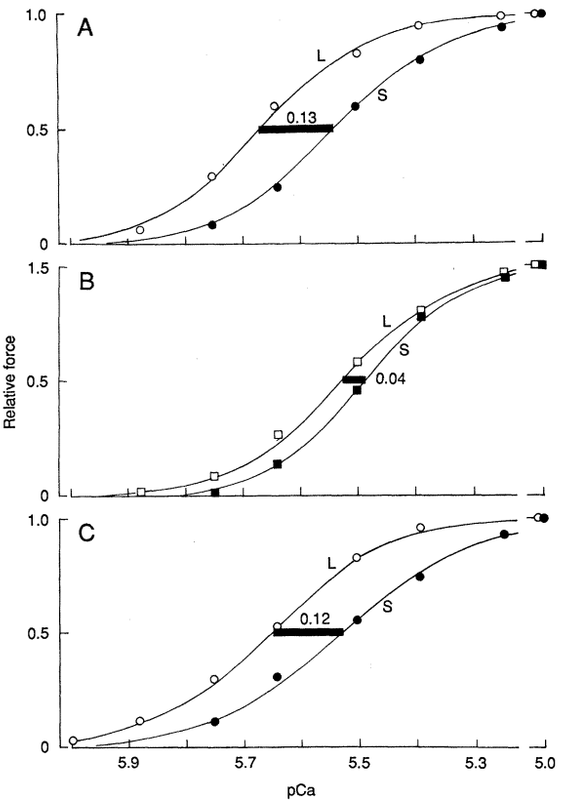

Fig. 2. Length dependence of the relation between pCa and force. L, 2.3 to 2.4 μm (open symbols); S, 1.9 μm (closed symbols). The first and last measurements were made at 2.4 μm. The data shown on three different trabeculae (**A**, **B**, and **C**) are typical. Between animals there was a small variation in the placement of the activation curve at 2.4 μm, but the net shift from 2.4 to 1.9 μm was similar in different preparations. The lines are computer fits of the Hill equation. The thick horizontal lines indicate the separation, and numbers show the shift in pCa units. The mean value ± SEM for five CTnC-type trabeculae was 0.128 ± 0.006 and for three STnC-loaded trabeculae was 0.020 ± 0.011 ($P < 0.001$ by t-test).

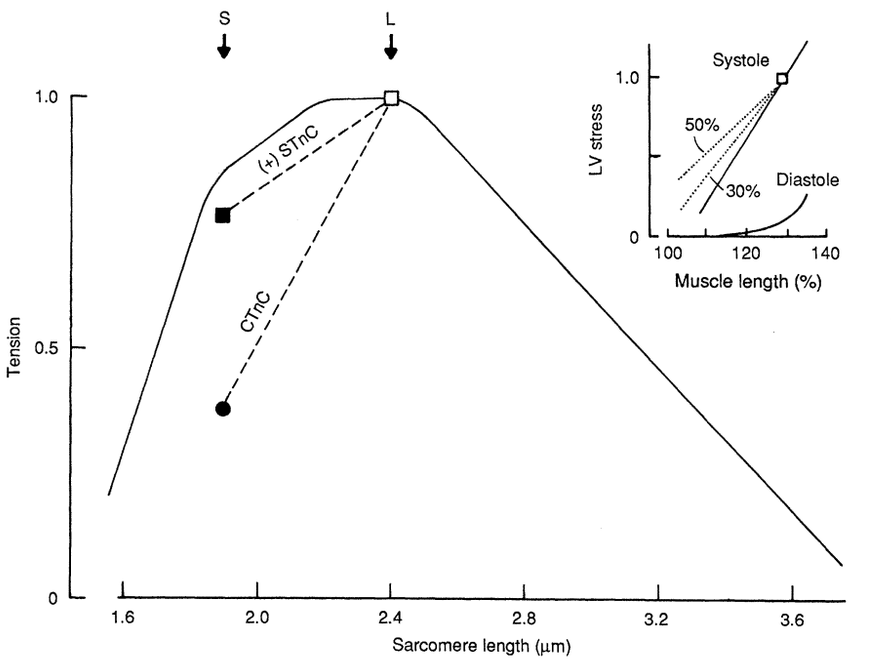

Fig. 3. The effect of type of TnC on length-tension relation of the partially activated cardiac muscle (50% activation level). The data points are normalized to the values at 2.4 μm. The solid line from previously published data (*25*) is translated to the mammalian fiber (*26*). (Inset) Left ventricular stress in systole and diastole from the data on dog heart (*19*). The 130% length, taken as 2.3 μm, is assumed to correspond to the point at the start of ejection in this case. The length-tension relations for 50% and 30% activations from Fig. 2 are superimposed.

(*20*) and 100 to 300 kN/m^2 as the maximum force-generating capability of the individual cells.

The TnC in cardiac muscle might respond differently from that in fast twitch skeletal muscle in several respects. One of the two trigger sites (the low-affinity Ca^{2+}-specific site I) in CTnC has 7 of the 12 amino acid residues replaced (*13*) in the Ca^{2+}-binding loop (*21*), modifying the Ca^{2+} coordination in the site under normal conditions, and this may be important in producing high length-induced Ca^{2+} sensitivity in cardiac muscle. There are also other minor replacements in the CTnC molecule, but their functional significance is unknown. How the length signal is transmitted to the TnC moiety in the cardiac muscle is also unknown. One possibility is that, as in skeletal muscle, a third set of filaments (containing titin, also called connectin) in the cytoskeletal matrix connects the myosin filaments to the Z-lines at the end of the sarcomere (*22*). The stress in the titin filament induced by sarcomere length would have to be communicated to the regulatory proteins if titin did play a specific role in the Starling mechanism. Alternatively, steric rearrangements within the myofilament lattice below 2.4 μm might selectively reorder the thin filaments in the heart muscle through the altered electrostatic or mechanical factors (*23*) and thereby also affect the regulatory proteins. Further investigations will be necessary to clarify these issues.

REFERENCES AND NOTES

1. O. Frank, *Z. Biol.* **32**, 370 (1895); E. H. Starling, in *Starling's Law of the Heart* (Longmans, Green, London, 1918).
2. R. J. Podolsky, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **21**, 964 (1962); B. R. Jewell, in *The Physiological Basis of the Starling's Law of the Heart* (Ciba Foundation Symposium, Elsevier, New York, 1974); E. Braunwald and J. Ross, *Circ. Res.* **15** (suppl. 2), 169 (1964).
3. D. G. Allen, B. R. Jewell, J. W. Murray, *Nature (London)* **248**, 606 (1984).
4. A. Fabiato and F. Fabiato, *ibid.* **256**, 54 (1975).
5. B. R. Jewell, *Circ. Res.* **40**, 221 (1977); M. I. M. Noble, *Clin. Sci. Mol. Mech.* **54**, 1 (1975).
6. D. G. Allen and J. C. Kentish, *J. Mol. Cell. Cardiol.* **17**, 821 (1985).
7. A. Babu, S. Scordilis, E. Sonnenblick, J. Gulati, *J. Biol. Chem.* **262**, 5815 (1987); A. Babu, S. Pemrick, J. Gulati, *FEBS Lett.* **203**, 20 (1986).
8. M. G. Hibberd and B. R. Jewell, *J. Physiol. (London)* **329**, 527 (1982).
9. J. C. Kentish *et al.*, *ibid.* **345**, 24P (1983).
10. There is variation in the data on Ca^{2+} sensitivity, but it is clear that the length dependence of the sensitivity is higher in the myocardium than in skeletal muscle. In frog ventricle a change of 0.4 μm in sarcomere length produced a shift of about 0.16 unit of pCa (*11*); in frog semitendinosus muscle fiber the corresponding shift amounted to 0.06 unit [see (*12*) for a review]. In rat myocardium, the pCa shift for half maximum activation was 0.21 unit (*8*), and the corresponding shift for fast twitch skeletal muscle fibers was 0.12 unit (*12*); but the length range was different (myocardium, 1.9 to 2.4 μm; skeletal muscle, 2.5 to 3.0 μm). Over the same range as used for fast twitch fibers (that is, 2.5 to 3.0 μm), slow fibers gave a shift of 0.30 pCa unit, nearly three times higher than in fast fibers (*12*). This is consistent with our findings, because the TnC of the slow fibers is nearly identical to CTnC, and both lack one Ca^{2+}-specific site (*13*).
11. A. Fabiato and F. Fabiato, *J. Gen. Physiol.* **72**, 667 (1978).
12. D. G. Stephenson and I. R. Wendt, *J. Muscle Res. Cell. Mot.* **5**, 243 (1984).
13. J. M. Wilkinson, *Eur. J. Biochem.* **103**, 179 (1980); P. Leavis and J. Gergely, *CRC Crit. Rev. Biochem.* **255**, 962 (1984); J. Potter and J. Johnson, *Calcium Cell Funct.* **2**, 145 (1982).
14. The extraction duration was purposely limited because we have shown that 100% TnC deletion from skeletal muscle causes the loss of a regulatory cofactor that prevents activation by Ca^{2+} in physiological salt solution even after the TnC is restored (*15*).
15. J. Gulati and A. Babu, *Biochem. Biophys. Res. Commun.* **151**, 170 (1988); *Biophys. J.* **53**, 23a (1988).
16. The converse experiment, which would show whether the skeletal muscle is made more sensitive to length change by substituting CTnC for STnC, is more difficult because the effectiveness of CTnC in reconstituting skeletal muscle is highly salt-dependent (*17*).
17. A. Babu and J. Gulati, in *Molecular Mechanisms of Muscle Contraction*, H. Sugi and G. Pollack, Eds. (Plenum, New York, 1988), p. 101; A. Babu *et al.*, *Biophys. J.* **53**, 589a (1988).
18. R. J. Podolsky, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1374 (1975).
19. K. Weber *et al.*, *Am. J. Physiol.* **231**, 337 (1976).
20. H. Badeer, *Am. Heart J.* **56**, 432 (1963); F. Yin, *Circ. Res.* **49**, 829 (1981).
21. Y. Babu *et al.*, *Nature (London)* **315** 37 (1985); R. H. Kretsinger, *CRC Crit. Rev. Biochem.* **8**, 119 (1980).
22. K. Wang, *Cell Muscle Mot.* **6**, 315 (1985); A. Magid *et al.*, in *Contractile Mechanisms in Muscle*, G. Pollack and H. Sugi, Eds. (Plenum, New York, 1984), p. 307; R. Horwitz and R. J. Podolsky, *J. Cell Biol.* **105**, 315 (1987).
23. T. Matsuda and R. J. Podolsky, *J. Mol. Biol.* **189**, 361 (1986).
24. J. Gulati and A. Babu, *J. Gen. Physiol.* **86**, 479 (1985).
25. A. Gordon, A. F. Huxley, F. Julian, *J. Physiol. (London)* **184**, 170 (1966).
26. H. E. Huxley, *J. Mol. Biol.* **7**, 281 (1963).

27. We thank S. Scordilis (Smith College) for suggestions and for purified TnCs, F.-C. A. Chiu (Neurology Department) for discussions, and A. Malhotra (Montefiore Hospital) for help in interpreting the gels. Supported by a grant from the National Institute of Arthritis, Musculoskeletal and Skin Diseases.

17 November 1987; accepted 4 February 1988

Cone Cell–Specific Genes Expressed in Retinoblastoma

E. BOGENMANN, M. A. LOCHRIE, M. I. SIMON

Retinoblastoma, an intraocular tumor that occurs in children, has long been regarded, on the basis of morphological criteria, as a malignancy of the photoreceptor cell lineage. Here it is shown that when this tumor is grown in vitro, the cells express highly specialized photoreceptor cell genes. Transcripts for the transducin alpha subunit, $T_{C\alpha}$, which is specific to the cone cell, as well as transcripts for the red or green cone cell photopigment, were found in seven out of seven low-passage retinoblastoma cell lines. No marker genes specific to rod cells were expressed, suggesting that retinoblastoma has a cone cell lineage.

RETINOBLASTOMA (RB) IS AN INTRAOCULAR tumor that occurs in children. The neoplasm develops early in life on a sporadic or hereditary basis and may be unilateral or bilateral, with the hereditary form being mainly bilateral (*1*). A chromosomal abnormality has been described for both forms of RB (*2*), and a gene in the RB locus was recently cloned (*3*).

The tumor is derived from neuroectodermal cells, but its cell of origin has not been unequivocally identified. Primary tumors are composed of anaplastic cells with little cytoplasm, and morphologically differentiated structures (fleurettes and Flexner-Wintersteiner rosettes) are found that express structural characteristics of mature photoreceptor cells (*4*). A bidirectional differentiation potential to photoreceptor cells or glial cells, or both, has been postulated on the basis of studies with the Y79 cell line (*5*).

Rod and cone cells are the photoreceptors of the mammalian retina. They express the photopigment genes (rhodopsin and color photopigments) (*6*) that have been isolated from the human genome (*7, 8*). Photopigment photolysis is coupled to cyclic guanosine 5′-phosphate metabolism through a membrane-associated retinal G protein, transducin (*9–11*), and results in a hyperpolarization of the photoreceptor cell membrane (*12, 13*). Bovine complementary DNAs (cDNAs) specific for the rod cell ($T_{R\alpha}$) and cone cell ($T_{C\alpha}$) alpha subunits of transducin have been isolated (*10, 14, 15*).

Here we show that cultured RB cell lines established from individual patients express the red or green photopigment gene, or both genes, but not the gene coding for rhodopsin. Concomitantly, the cells express the cone cell $T_{C\alpha}$ subunit but not the rod cell $T_{R\alpha}$ subunit of transducin. We therefore postulate that RB may be a neoplasm of the cone cell lineage.

Previously isolated RB cell lines that show

E. Bogenmann, Division of Hematology-Oncology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027.
M. A. Lochrie and M. I. Simon, Division of Biology, California Institute of Technology, Pasadena, CA 91125.