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Titin and the Developing Heart

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During gestation, the mammalian heart operates under very different loading conditions than those seen during adult life. Ensuring that cardiac output is sufficient at the low filling pressures found in the fetal circulation requires mechanical strategies and protein complements different from those seen in the adult.

The studies by Opitz et al,[1] in this issue of *Circulation Research*, and Lahmers et al,[2] published recently in this journal, provide some clues as to the molecular basis of these strategies. Both studies examined the developing heart and found correlations between the expression of titin isoforms and the increased stiffness of the myocardium as the organism progressed from fetus through neonate to adult.

The pattern of findings is remarkably consistent across both studies and, despite differences with earlier claims of a less compliant fetal heart, leads to the conclusion that the fetal sarcomere is more compliant than the adult and that this is in large part due to the expression of particular isoforms of titin. But why is a more compliant titin isoform beneficial to the fetus?

Titin

We will briefly review the properties of titin here. Primary sources may be found by consulting recent reviews.[3–9]

Titin, also known as connectin, is a relatively recently discovered giant protein (3 to 4 MDa) that is the third most abundant protein in striated muscle, forming up to 10% of the total protein content of the cell. Titin extends half the length of the sarcomere from the Z-disc through the I-band and A-band to the M-line, a distance of about 1 μm.[7]

The N-terminal end of titin is capped by telethonin, a 19-kDa protein that appears to be confined to striated muscle and that may have a role in organizing myofibrillogenesis.[10–12] The N-terminal region of titin closest to the Z-disc interacts with actin and α-actinin via 45 residue domains termed Z-repeats; cardiac muscle has seven of these Z-repeats that each span 38.5 nm.[10,13] At the M-line, the C-terminal end of titin interacts with myosin binding protein C (MyBP-C).

Titin arises from a single gene that has 363 exons (human). These exons give rise to more than 38 000 residues. Over a third of these exons offer alternative splices to produce a variety of titin isoforms. The first 251 exons code for regions of titin that lie within the sarcomere's I-band; the remaining 112 exons code for regions in the A-band. A serine-threonine kinase domain near the C-terminal that is able to phosphorylate telethonin is coded for by exon 358.

The elasticity of titin originates within the portion lying in the I-band and is due to the presence in that region of (1) tandem immunoglobulin (Ig) repeats, (2) a region rich in proline (P), glutamate (E), valine (V), and lysine (K) arranged in 28 residue repeats; the PEVK region and (3) a region with a sequence unique to titin, the N2B region. Alternative splicing provides additional tandem Ig repeats that insert between the N2B and PEVK regions. These longer titin isoforms also have an N2A region and are termed the N2BA isotype.

In a sarcomere at slack length, titin is highly folded with many of the regions acting as entropic springs. As the sarcomere is stretched, the links between the tandem Ig repeats extend first. As the stretch proceeds, the PEVK sequence unfolds and at the upper end of the physiological range of sarcomere lengths the N2B region also unfolds. Over the normal physiological range of sarcomere lengths and forces, the tandem Ig repeats are *not* thought to unfold although these repeats can be made to unfold in isolated titin molecules.[3,14]

Several molecular models have been proposed to explain the titin stress-length relation within the range of lengths seen in the sarcomere. The worm-like-chain model seems to have an advantage in explaining titin's elasticity over the full range of lengths and consequently is commonly used to describe titin's behavior over physiological sarcomere lengths.[14]

The Present Studies

Both studies show evidence of a previously unknown isoform of titin, termed by Opitz et al[1] as N2BA-1. Optiz et al suggest that this novel isoform is dominant in the fetus and almost absent by birth in the rat. Lahmers et al[2] also found that the rabbit has high levels of N2BA-1 at birth but that these levels fall rapidly (half-life of about 7 days). Lahmers et al[2] found that the N2BA isoform in the adult appeared to have a different mobility suggesting that the N2BA has three isoforms. Both studies agree that the dominant isoform in the adult animal is the shorter and stiffer N2B. The pig differs from the rat and rabbit in having a significant level of N2BA in the adult myocardium. Nevertheless, both groups showed a reduction in the ratio N2BA:N2B as the animal developed from neonate to adult.

Immunoelectromicroscopy studies by Lahmers et al[2] showed the N2BA isoform to be within the sarcomere. Differences between fetus and adult in the position of an antibody marking a common epitope while the sarcomere was under tension provided additional evidence that the adult N2BA isoform differed from the fetal isoforms.

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(*Circ Res.* 2004;94:860-862.)

***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000126698.37440.B0

Both groups noted that fetal and neonatal levels of the T2 degradation product of titin were higher than in the adult. This might be expected with a higher turnover of titin in early development.

Optiz et al[1] examined the expression of titin using the quantitative reverse transcriptase polymerase chain reaction. They showed little change in total titin message level from fetal day 19 to adulthood. Somewhat surprisingly, the N2BA-mRNA levels remained high despite significant variations in protein levels during development and the near absence of the N2BA isoforms in the adult. This intriguing observation suggests that longer titin mRNAs may be less stable and have a lower translation efficiency, or that the longer titin isoforms themselves may be less stable. Alternatively, Opitz et al[1] suggest that this discrepancy between mRNA and protein levels may be due to the existence of multiple N2BA-mRNA's each for a different splice variant. The variants may not be readily distinguishable as protein isoforms in gel separations.

RT-PCR showed a change in message for N2B isoforms that increased with development, peaking at postnatal day 16 and declining slightly in adulthood. This appears to correlate reasonably with changes in N2B protein levels.

Lahmers et al[2] used a microarray that included all 363 titin exons. In the fetus, some 20 exons were upregulated with the majority coding for additional Ig domains in the I27–I79 splice region or domains in the PEVK region. This suggests that fetal titin isoforms would be more compliant than the adult isoforms.

Both groups examined the role of titin in the passive stiffness of myocardial tissue, Opitz et al[1] in rat myofibrils, Lahmers et al[2] in skinned swine muscle strips. Lahmers et al measured passive tension as a product of a ramp stretch at 1 s^{-1} from slack length (sarcomere length [SL] of about 1.9 μm) to a sarcomere length of at least 2.4 μm. Opitz et al used a step increase in length and held the length until force stabilized. This was repeated from an SL of about 1.7 μm to more than 3.5 μm. The differences in technique produced some discrepancies in absolute values for the passive tension, but both studies showed higher values in the adult than in the fetus.

Altered Compliance of Fetal Heart

Early studies suggested that fetal heart is less compliant than the adult heart[15] and indeed this seems to be accepted in the literature.[16] Studies on isolated hearts also found fetal hearts to be *less* compliant than adult hearts.[17] Likewise, studies on ventricular moderator bands from fetal and newborn lambs and from adult sheep also found fetal cardiac muscle strips to be *less* compliant than strips from adults.[18] Similar results were found for cats where neonatal papillary muscles were stiffer than adult muscles.[19]

This contrasts with the current work of Opitz et al[1] and Lahmer et al.[2] However, the early work was done in larger intact muscle bundle preparations instead of skinned fibers and myofibrils. Muscle lengths were normalized to the length of maximum developed force rather than using sarcomere lengths. Other technical issues may also have led to different conclusions, eg, different species, proportion of contractile material in cross section, and the possibility of hypoxic regions in larger preparations. It would be useful to reexamine the compliance of fetal heart, from myofibril to intact chamber, to determine if the scale on which the compliance is measured has an effect on the results obtained.

Why Is a More Compliant Sarcomere Beneficial for the Fetus?

What is the role of a more compliant sarcomere in the performance and development of the fetal heart given that it is subject to external constraint by the pericardium and chest wall?

A simple answer is that the low filling pressure (end-diastolic pressure of about 2.5 to 8 mm Hg) requires the fetal heart to be more compliant to reach sarcomere lengths needed to develop sufficient tension to pump. However, this answer fails to take into account the constraint imposed on the fetal heart by the surrounding tissues. The fetus has a limited cardiac reserve due in large part to this constraint,[16,20,21] and it may be that this constraint also limits the functional effect of a more compliant sarcomere.

Another possible role for compliant titin isoforms may be in myofibrillogenesis in the growing fetus. Obscurin is also upregulated during early development,[1] a titin-like Z-disc–associated protein strategically located in the link to the costamere.[10] It is tempting to speculate that both proteins play an important role in the signaling pathway regulating sarcomere assembly in response to stretch. That is, the fetal heart undergoes significant enlargement during development and this requires addition of sarcomeres both in series and in parallel. The mechanism for this is poorly understood. In cultured neonatal rat cardiac myocytes subject to mechanical strain, sarcomeres can be added in series within 4 to 6 hours.[22] This is in direct response to strain of the myocytes and does not necessarily represent normal growth patterns. Nevertheless, it may be that the more compliant fetal titin plays a role in the development of the heart by co-opting the molecular machinery that builds sarcomeres in response to stretch. In that light, it is of interest to note that alterations in titin isoform profile have been found in experimental and human heart failure,[23,24] and that some mutations in titin have been associated with human cardiomyopathies.[25] Hence, apart from its role in regulating sarcomere compliance, titin may also play a pivotal role in regulating sarcomere homeostasis in response to a variety of loading conditions imposed on the heart.

Acknowledgments

This work was supported in part by NIH PO1 HL-62426 (project 4).

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KEY WORDS: connectin ■ compliance ■ fetal ■ neonatal