Published in final edited form as:

J Mol Cell Cardiol. 2008 June ; 44(6): 976–978.

SOME RAT: A VERY SPECIAL RAT WITH A RATHER SPECIAL TITIN

Olivier Cazorla¹ and Pieter P. de Tombe²

¹ Center for Cardiovascular Research, Department of Physiology and Biophysics M/C 901, University of Illinois at Chicago, College of Medicine Chicago, IL, 60612, USA

² INSERM, U 637, F-34925 Montpellier, France. Université MONTPELLIER, UFR de Médecine, F-34925 Montpellier, France

Cycling cross-bridges, the molecular interactions between the contractile proteins actin and myosin, generate the muscle force that underlies cardiac pressure development. In striated muscle, these proteins are organized in the thick and thin filaments that together form the sarcomere. A more recent discovery localizes a third filament to the sarcomere, one that is composed of titin. Titin, also known as connectin, is a giant protein (3–4 MDaltons) that is an abundant protein in striated muscle forming up to 10% of the total protein content of the cardiac cell. Titin extends half the length of the sarcomere from the Z-disc through the I-band and A-band on to the M-line (~1 μ m). The molecule is tightly anchored at its NH₂-terminus in the Z-disk via interactions with alpha-actinin and at its C-terminal domain to the M-line via interactions with myosin. The cell biology of titin has been the subject of intense study by several groups following its discovery in 1979[1]. It was immediately recognized that a significant function of titin in the sarcomere might be structural. Indeed, titin has emerged as the main cellular structure responsible for passive striated muscle cell stiffness[2,3]. Furthermore, within the physiological range of cardiac volume, titin appears to be responsible for a significant portion of the diastolic passive filling pressure of the heart; the remainder of the elastic force being generated by extracellular matrix collagen[4]. The elasticity of titin originates within the I-band portion due to the presence of *i*) tandem immunoglobulin (Ig) repeats, *ii*) a region rich in proline (P), glutamate (E), valine (V), and lysine (K), the PEVK region and *iii*) a region with a sequence unique to cardiac 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 N2BA. 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[2,3].

It has become increasingly clear recently that titin's functions are far more complex and that this giant molecule may play a much larger role in striated muscle physiology than merely as a passive stress bearing protein. For example, it has been suggested that titin plays an important

Correspondence: Pieter P. de Tombe, Ph.D., Center for Cardiovascular Research, University of Illinois at Chicago, Department of Physiology and Biophysics MC901, 835 S. Wolcott Ave, Chicago, IL 60612, USA, Phone: +1-312-355-0259, FAX: +1-312-355-0261, Email: pdetombe@uic.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

role in regulating both protein turnover as well as regulating gene expression in response to mechanical strain[2,3]. Moreover, the protein itself is a kinase capable, at least in-vitro, of phosphorylating telethonin (T-cap), a titin capping protein located at the Z-disk region of titin. However, whether this actually occurs in vivo is not clear since titin's kinase domain is physically far removed from the T-cap substrate. Nevertheless, the kinase is ideally positioned to sense mechanical strain, thus constituting a potential molecular mechanism by which various cellular processes may be regulated (for example gene expression and/or protein turnover[2, 3,5]). Indeed, mutations in this region have been linked to hereditary muscle disease underscoring its importance in cellular biology[6]. Additionally, it has been proposed that titin may be a molecular ruler, setting the size of the thick filament during sarcomere assembly[7]. Consistent with this notion, knock-out of titin's M-line binding region leads to a general failure to assemble sarcomeres during embryonic development[8,9]. Finally, titin has been identified as a substrate for protein kinase A (PKA) in the heart. PKA mediated phosphorylation of titin results in decreased stiffness of the cardiac myofilament. This process may be physiologically significant as it was recently shown that myocytes obtained from patients with diastolic heart failure were less compliant than normal cells and treatment of the skinned myocytes with PKA reversed this abnormality[10].

The human genome is estimated to code for 30,000–40,000 genes. This number is much less than the number of unique expressed sequence tags that suggest an estimate of 100,000–150,000 unique proteins that may be expressed in human cells; this number further rises due to distinct post-translational modifications[11]. A main mechanism by which this occurs is via alternative splicing of the genomic copy in the form of pre-messenger RNA into various spliced RNA products that can *i*) vary in their protein coding region to alter the amino-acid sequence and, thus, protein function, or *ii*) vary in the non-coding regions to alter RNA stability and/or translational efficiency[12]. RNA splicing appears to be a tightly regulated cellular process that takes place in specialized structures called spliceosomes; the mechanisms underlying these processes are incompletely understood. Many proteins, perhaps up to 75%, are subject to alternative splicing, and titin is no exception. Titin arises from a single gene that has ~350 exons giving rise to ~40,000 residues, equivalent to a titin molecule of ~4MD! This is a giant molecule indeed, possibly the largest protein in the mammalian cell known to date. The remarkable diversity of titin molecules found in muscles (and in some other cell types as well) is the result of alternative splice products from over a third of these exons[2,3]. During gestation, fetal development, and ageing to adulthood, the mammalian heart has been shown to express titin isoforms that become ever shorter with age via the mechanism of alternative splicing. Consistent with the proposed mechanical role for titin, cardiac myocyte cellular stiffness increases with age in tight correlation with the expressed titin isoform distribution. In addition, several cardiac pathologies are associated with a re-expression of a longer titin isoform and a concomitant reduction in cardiac myocyte passive stiffness[2,3]. Whether reduced passive stiffness of the diseased heart is beneficial or detrimental to cardiac function is not known, nor are the molecular mechanisms that underlie titin isoform switching. A recent study by Kruger et al using primary cardiac myocytes in culture suggest that the titin isoform switch is not causally related to myocyte hypertrophy per se, but rather via a thyroid sensitive pathway that may include PI3K/Akt-dependent signaling[13].

A difficulty associated with the study of a giant structural protein such as titin, is genetic manipulation of expression levels to alter cellular function. For example, the protein is certainly too large to be packaged into adenoviral constructs, let alone adeno-associated virus. Due to its role in sarcomere assembly during embryonic development, knockout of (portions of) titin often leads to lethality, prompting the use of conditional knockout models, e.g. [8]. Moreover, not only the sheer size of the protein, but also the large number of potential protein binding partners thus far identified (and that list is certain to grow in the future) makes deciphering the physiological roles of titin a daunting task[2,3]. In this current issue, Dr. Greaser and his co-

workers introduce a novel and rather unusual tool to the arsenal of investigative tools for the study of titin physiology[14]. Rather than attempting to genetically alter titin expression in transgenic mice, these investigators have cleverly capitalized on an apparent spontaneous titin mutation which they serendipitously discovered in a litter of Sprague-Dawley rats during the course of a study on the alternative splicing of titin during development[15]. As mentioned above, the expressed isoform of titin generally becomes shorter with ageing, and this also occurs in the rat[2,3]. However, a few animals in the earlier study displayed a marked delay in the expression of the shorter isoform; in the study reported here, the “aberrant” rats have been bred into a homozygous strain, all expressing a giant form of titin (3.9 MD!) and, moreover, these rats appeared not to undergo the usual age transitions where the shorter titin isoform becomes the dominant isoform in adulthood. The transition was not altogether aborted because the shorter N2B isoform is expressed in the hearts of aged rats of this mutant strain, but at a level far below normal. Importantly, the isoform profile of troponin-T, another developmentally regulated contractile protein, was perfectly normal. This finding lends strong support for the notion that this rat strain does not just suffer from a general derangement of alternative RNA splicing, but rather this mutation specifically affects titin RNA splicing biology. There are several aspects of the study by Greaser et al that are particularly exciting. First, this mutant strain of rats will allow an in depth investigation of the physiological mechanical role of titin in the heart. As a first step towards this goal, physiological examination of the passive stress-strain relation revealed a much more compliant cardiac myocyte that could be stretched to a sarcomere length well beyond filament overlap (~4 μm). The importance of this finding cannot be overstated; usually, cardiac sarcomeres cannot be stretched much beyond ~2.5 μm , and contractions can only be studied in mammalian myocardium, for the most part, at a sarcomere length where double actin filament overlap occurs[16]. With this mutant rat strain, however, mechanical studies can now be performed over a much wider sarcomere length range, at least in single isolated myocytes. Incidentally, having an unusually long titin isoform in the heart and a very compliant myofilament appeared not to be detrimental to the mutant rats, suggestive of a well-compensated hemodynamic state; this may well have been the result of a compensatory general stiffening of the extra-cellular matrix. Second, the role of titin as length sensing molecule can now be studied in great detail. Of interest, the myocytes in the hearts of the mutant rats were considerably longer, as was resting sarcomere length. This result either points to the role of titin as “molecular ruler”[7] or it may indicate that the “strain signal” transmitted via titin was much reduced in the mutant hearts leading to an alteration in strain dependent protein expression and sarcomere assembly signaling[2,3,5]. Clearly, the availability of this mutant strain of rats opens the door to comprehensive genomic/proteomics studies in which the impact of strain is compared between wild-type and mutant rat myocardium. Yet another form of length sensing in the heart is the Frank-Starling mechanism, a property of the heart that is caused by a modulation of myofilament Ca^{2+} sensitivity in response to changes in sarcomere length[16,17]. Although the mechanisms underlying myofilament length dependent activation are not well understood[17], titin has been implicated to form part of the length sensing apparatus of the sarcomere[18,19]; the mutant rats will now allow for a more direct test of this hypothesis. Third, and perhaps the most exciting aspect of the study by Greaser et al, are the possibilities that this mutant rat strain offers to the study of the mechanisms that underlie alternative splicing of titin and, perhaps, alternative splicing in general. That is, a differential proteomic comparison between wild-type and mutant myocardium in health and disease is very likely to reveal important clues to unravel which cellular signals are orchestrating the remarkable diversity of titin molecules that are expressed in the mammalian heart. Hence, even though this rat may not be able to read or write, there is no doubt that in the very near future it *will* be able to tell quite an exciting story (or many), earning it the well deserved status of “some rat” indeed!

Acknowledgements

Supported NIH grants HL-62426, HL-75494, HL-77195; a Leducq Trans Atlantic Network Award; the Association Française contre les Myopathies; a Région Languedoc-Roussillon Award. OC and AL are established investigators of CNRS, PPT was supported by INSERM.

References

1. Wang K, McClure J, Tu A. Titin: major myofibrillar components of striated muscle. *Proceedings of the National Academy of Sciences of the United States of America* 1979 Aug;76(8):3698–702. [PubMed: 291034]
2. Granzier HL, Labeit S. The giant protein titin: a major player in myocardial mechanics, signaling, and disease. *Circulation research* 2004 Feb 20;94(3):284–95. [PubMed: 14976139]
3. Linke WA. Sense and stretchability: The role of titin and titin-associated proteins in myocardial stress-sensing and mechanical dysfunction. *Cardiovasc Res* 2008 Mar 1;77(4):637–48. [PubMed: 17475230]
4. Granzier HL, Irving TC. Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. *Biophys J* 1995;68(3):1027–44. [PubMed: 7756523]
5. Lange S, Xiang F, Yakovenko A, Vihola A, Hackman P, Rostkova E, et al. The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 2005 Jun 10;308(5728):1599–603. [PubMed: 15802564]
6. Carmignac V, Salih MA, Quijano-Roy S, Marchand S, Al Rayess MM, Mukhtar MM, et al. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann Neurol* 2007 Apr; 61(4):340–51. [PubMed: 17444505]
7. Trinick J. Titin and nebulin: protein rulers in muscle? *Trends Biochem Sci* 1994 Oct;19(10):405–9. [PubMed: 7817397]
8. Peng J, Raddatz K, Molkenin JD, Wu Y, Labeit S, Granzier H, et al. Cardiac hypertrophy and reduced contractility in hearts deficient in the titin kinase region. *Circulation* 2007 Feb 13;115(6):743–51. [PubMed: 17261657]
9. Weinert S, Bergmann N, Luo X, Erdmann B, Gotthardt M. M line-deficient titin causes cardiac lethality through impaired maturation of the sarcomere. *J Cell Biol* 2006 May 22;173(4):559–70. [PubMed: 16702235]
10. Hamdani N, Kooij V, van Dijk S, Merkus D, Paulus WJ, Dos Remedios C, et al. Sarcomeric dysfunction in heart failure. *Cardiovasc Res.* 2008 Jan 11;
11. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. *Science* 2001 Feb 16;291(5507):1304–51. [PubMed: 11181995]
12. Lopez AJ. Alternative splicing of pre-mRNA: developmental consequences and mechanisms of regulation. *Annu Rev Genet* 1998;32:279–305. [PubMed: 9928482]
13. Kruger M, Sachse C, Zimmermann WH, Eschenhagen T, Klede S, Linke WA. Thyroid hormone regulates developmental titin isoform transitions via the phosphatidylinositol-3-kinase/AKT pathway. *Circulation research* 2008 Feb 29;102(4):439–47. [PubMed: 18096819]
14. Greaser ML, Warren CM, Esbona K, Guo W, Duan Y, Parrish M, et al. Mutation that dramatically alters rat titin isoform expression and cardiomyocyte passive tension. *Journal of Cellular and Molecular Cardiology.* 2008in press
15. Greaser ML, Krzesinski PR, Warren CM, Kirkpatrick B, Campbell KS, Moss RL. Developmental changes in rat cardiac titin/connectin: transitions in normal animals and in mutants with a delayed pattern of isoform transition. *Journal of muscle research and cell motility* 2005;26(6–8):325–32. [PubMed: 16491431]
16. de Tombe PP. Cardiac myofilaments: mechanics and regulation. *J Biomech* 2003 May;36(5):721–30. [PubMed: 12695002]
17. Konhilas JP, Irving TC, De Tombe PP. Frank-Starling law of the heart and the cellular mechanisms of length-dependent activation. *Pflugers Arch* 2002 Dec;445(3):305–10. [PubMed: 12466931]
18. Cazorla O, Vassort G, Garnier D, Le Guennec JY. Length modulation of active force in rat cardiac myocytes: is titin the sensor? *Journal of molecular and cellular cardiology* 1999;31(6):1215–27. [PubMed: 10371696]

19. Cazorla O, Wu Y, Irving TC, Granzier H. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circulation research* 2001 May 25;88(10):1028–35. [PubMed: 11375272]