

Triadin regulates cardiac muscle couplon structure and microdomain Ca^{2+} signalling: a path towards ventricular arrhythmias

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Abstract

Since the discovery of triadin >20 years ago as one of the major proteins located in the junctional sarcoplasmic reticulum, the field has come a long way in understanding the pivotal role of triadin in orchestrating sarcoplasmic reticulum Ca^{2+} -release and hence excitation–contraction (EC) coupling. Building on the information gathered from earlier lipid bilayer and myocyte overexpression studies, the gene-targeted ablation of *Trdn* demonstrated triadin's indispensable role for maintaining the structural integrity of the couplon. More recently, the discovery of inherited and acquired diseases displaying altered expression and function of triadin has further emphasized the role of triadin in health and disease. Novel therapeutic approaches could be aimed at correcting the loss of triadin in diseased hearts, and thereby correcting the sub-cellular EC coupling defect. This review summarizes current concepts of the impact of triadin on cardiac EC coupling with a focus towards triadin's role for ventricular arrhythmia.

Keywords

Cardiac calcium handling • Ventricular arrhythmia • Cardiac couplon ultrastructure • Triadin

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1. Introduction

In the heart, triadin and junctin are two specialized proteins that link the Ca^{2+} -buffering protein calsequestrin (Casq2) to the sarcoplasmic reticulum (SR) ryanodine receptor Ca^{2+} -release channels (RyR2) in the junctional SR (jSR).¹ In the jSR, RyR2s are in turn juxtaposed to L-type Ca^{2+} channels located on the sarcolemma-forming couplons which are the Ca^{2+} -release units (CRUs) pivotal to cardiac excitation–contraction (EC) coupling (Figure 1).

Triadin was discovered in the rabbit skeletal muscle more than 20 years ago.² Subsequently, its presence was also demonstrated in the heart³ where it exists in three isoforms.¹ The predominant cardiac isoform is the 35-kDa protein triadin-1.⁴ Additionally, two other isoforms with larger molecular weight are detected in minor quantities^{4,5} which may be due to non-specific binding of the antibody since they are also detected in murine hearts with gene-targeted ablation of *Trdn*.⁶ This review summarizes the role of triadin for maintaining couplon ultrastructure and healthy EC coupling and triadin's impact on microdomain Ca^{2+} signalling in the jSR with a focus towards ventricular arrhythmia. For the purpose of this review, the cardiac isoform of triadin CT-1 is simply referred to as triadin.

2. Co-expression of jSR proteins is highly regulated and well co-ordinated

The human triadin gene *TRDN* is fragmented and located on chromosome 6.⁷ It covers 420 kb and comprises at least 41 exons, with an average of 58 bp per exon.⁷ The isoforms of triadin are derived from a single gene by alternative splicing and are tissue specific.⁷ It is now known that splicing factor SRp38 directly regulates alternative splicing of triadin and that its loss leads to changes in triadin pre-mRNA splicing, and altered intracellular Ca^{2+} handling.⁸ Interestingly, SRp38 'knock-out' also down-regulates Casq2 mRNA and protein expression but does not affect other SR proteins indicating tightly co-ordinated co-expression of SR Ca^{2+} -handling proteins during cardiac development.⁸ Furthermore, there is growing evidence that additional regulator proteins such as the tail-anchored membrane proteins from the myocardium known as the sarcolemmal membrane-associated proteins⁹ or the transcription factor early growth response-1¹⁰ are involved in orchestrating SR Ca^{2+} -handling proteins co-expression and hence Ca^{2+} dynamics.

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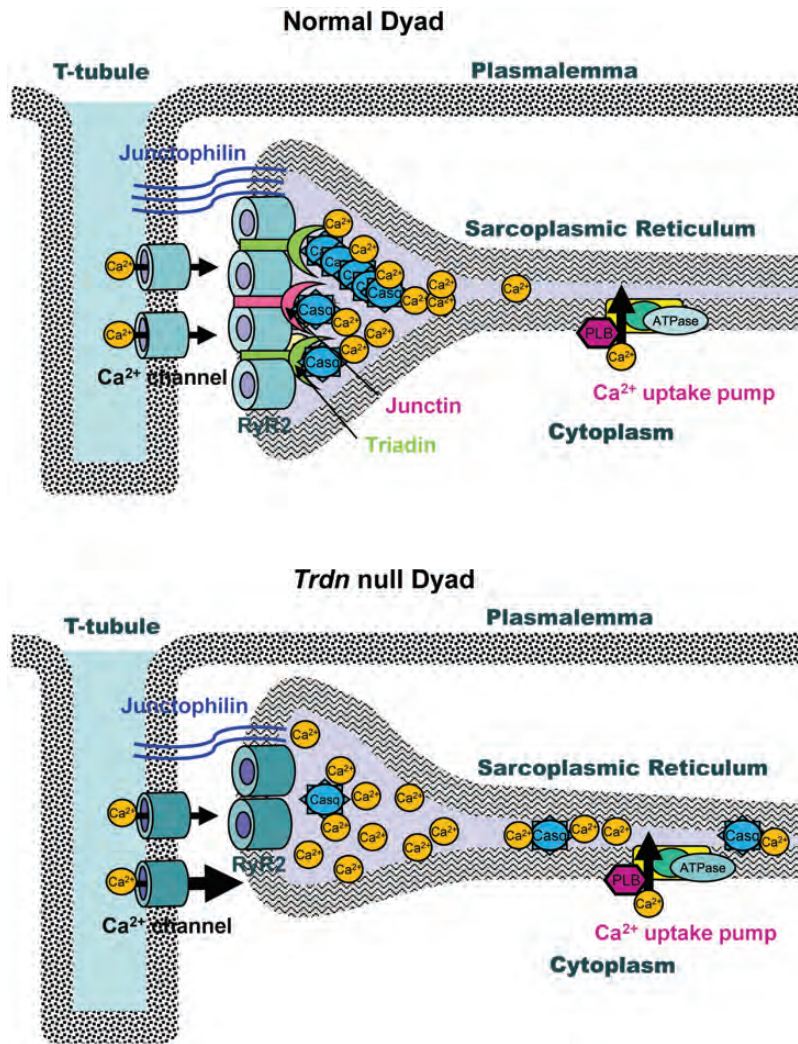


Figure 1 Cartoon illustrating the structure and protein composition of the dyads in normal and *Trdn* null cardiomyocytes.

The interdependence of expression of jSR Ca²⁺-handling proteins is evident from mouse models with gene-targeted ablation of either *Casq2* or *Trdn*. In both models, knockout of *Casq2* or *Trdn* causes a near-complete loss of the associated proteins *Casq2*, *junctin*, and *triadin*, suggesting that *in vivo* the three proteins form a protein complex.^{6,11} Notably, *junctin* requires *Casq2* and *triadin* for its expression but its gene-targeted ablation does not down-regulate the expression of the latter two proteins, suggesting that *junctin*'s role for stability of the jSR protein complex is redundant.¹² However, it is now known that despite sharing a close homology with *triadin*, *junctin* serves a different function than *triadin*. *Junctin* directly regulates SR Ca²⁺ release without affecting the expression of related jSR Ca²⁺-handling proteins or the architecture of the couplon.¹³ *Junctin* seems to have a dual effect on the response of RyR2s to luminal Ca²⁺.¹³ At low luminal Ca²⁺ (<1 mmol/L), *junctin*'s loss attenuates SR Ca²⁺ release; conversely, at high luminal Ca²⁺ (i.e. with β -adrenergic stimulation) *junctin*'s absence sensitizes the RyR2s and increases SR Ca²⁺ leak.¹³ However, this dual effect

may be related to regulation of RyR2 by *Casq2* and *triadin* in the absence of *junctin* which cannot be entirely ruled out.¹⁴

3. Why study triadin in cardiac muscle?

Although the structure of *triadin* has extensively been studied,^{1,15} *triadin*'s function in the heart still remains to be fully elucidated.¹² In humans, both missense and nonsense mutations in *TRDN* have recently been linked to a genetic form of ventricular arrhythmia and sudden cardiac death—catecholaminergic polymorphic ventricular tachycardia (CPVT, discussed in detail below).¹⁶ Overexpression of *junctin* in murine hearts was shown to increase susceptibility to atrial fibrillation.¹⁷ Down-regulation of *triadin* protein has been demonstrated in human heart failure, the significance of which is largely unknown.¹⁸ Furthermore, there are preliminary *in vitro* data showing impaired contractile recovery and increased injury following myocardial ischaemia

in the absence of the jSR proteins, junctin, or triadin.¹⁹ The aforementioned discoveries highlight the need for a better understanding of the role of triadin in healthy and diseased cardiac muscle.

4. Triadin sensitizes RyR2 in lipid bilayer and overexpression studies

The earliest functional studies showed that triadin protein sensitizes RyR2 channels and enhances SR Ca^{2+} release when applied to purified RyR2 channels incorporated in lipid bilayers.²⁰ However, when applied together with Casq2 and junctin, it may regulate RyR2 open probability in response to increasing luminal Ca^{2+} . This finding has prompted investigators to postulate that triadin together with Casq2 and junctin functions as a hetero-trimeric 'luminal Ca^{2+} sensor',²⁰ a notion that has remained controversial.^{12,21} Triadin also sensitizes the RyR2 when overexpressed using adenovirus-mediated gene transfer in isolated rat myocytes.¹⁴ Interestingly, gene-targeted α -myosin heavy-chain promoter-driven overexpression of triadin in murine hearts causes depressed contractility, elevated diastolic Ca^{2+} with slower Ca^{2+} transient decay *without* affecting SR Ca^{2+} release.²² These conflicting data suggest the role of triadin in EC coupling to be multifaceted, and therefore the function of *native* triadin in EC coupling in intact myocytes still remains largely unknown.

5. Triadin maintains the structural and hence functional integrity of the couplon and is required for normal EC coupling

To address the physiological role of triadin in cardiac muscle, our group used a gene-targeted ablation approach and studied the phenotype of *Trdn* null mice.⁶ Interestingly, as one would not have predicted from *in vitro* or overexpression studies, triadin appears to be required for maintaining the structural and *hence* functional integrity of the couplon, both being tightly interdependent to each other (*Figure 1*).

Ablation of *Trdn* is not lethal and the mice survive to old age with survival rates similar to that of wild-type littermates.⁶ In *Trdn* null hearts, there is reduced expression of the jSR proteins RyR2 (50%), Casq2 (~60%), junctin (~92%), and junctophilin 1 and 2 (~30–40%) without any significant change in the non-jSR proteins: SERCA2, phospholamban (*Figure 1*).⁶ Expression of L-type Ca^{2+} channel protein was unchanged.⁶ Since this change occurred at the level of the jSR proteins but not the mRNA, these data suggest that triadin somehow regulates either stability, targeting, or retention of binding partner proteins in the jSR. This is also supported by the finding that *trdn* null myocytes retain only a small amount of Casq2 in jSR cisternae with a significant fraction of the protein escaping and localizing in the free SR (*Figure 1*). Hence, a major role of triadin in cardiac muscle is that of a critical anchoring protein that links Casq2 to RyR2 and thereby retains Casq2 in the jSR.⁶

The deletion of triadin also causes a significant remodelling of the structure of the couplon such that there is overall 50% reduction in the close association between jSR and T-tubules, without any change in SR volume (*Figure 1*).⁶ This is evident from the electron microscopy of the dyads and co-localization studies using immunolabelling with anti-RyR2 antibody and anti-L-type Ca^{2+} channel antibody in isolated myocytes lacking triadin. An important consequence of the

couplon remodelling is that ~50% of L-type Ca^{2+} channels are not juxtaposed to RyR2 SR Ca^{2+} release channels. The alteration of couplon architecture is not unique to *Trdn* deletion since *Casq2* deletion increases SR volume by ~50%¹¹ suggesting their pivotal role in maintaining the architecture of the couplon (unlike junctin¹³) besides being instrumental in executing SR Ca^{2+} release. Interestingly, α -myosin heavy-chain promoter-induced expression of missense Casq2 mutation D307H in the *Casq2 null* background partially restores the ultrastructural changes reported with gene-targeted ablation of *Casq2*,¹¹ reiterating that the presence of Casq2 (even if mutated) is critical for maintaining the normal jSR architecture.²³ It is unclear how either triadin or Casq2, both of which are entirely confined to the membrane and/or lumen of jSR, would be able to stabilize the formation of T-tubule jSR interface. A possible explanation comes from the observation that junctophilin 1 and 2, proteins that have two membrane-spanning domains and likely serve as anchors for the T-tubule jSR couplon,²⁴ are both down-regulated in the triadin null hearts (*Figure 1*). Thus, it is intriguing to speculate that triadin may be required for either targeting or stabilizing junctophilin in the jSR.

The paucity of RyR2 and L-type Ca^{2+} channel juxtapositions in *Trdn* null myocytes has important functional consequences. There is an increase in SR Ca^{2+} load resulting from uninhibited Ca^{2+} influx via L-type Ca^{2+} channels due to the loss of negative feedback of SR Ca^{2+} release on L-type Ca^{2+} currents (*Figure 1*).⁶ On the other hand, Ca^{2+} -induced SR Ca^{2+} release is significantly reduced despite larger SR loads.⁶ Loss of junctin probably contributes to the attenuation of SR Ca^{2+} release.¹³ However, the mismatch of RyR2s and L-type Ca^{2+} channels appears to be the major contributor, since blocking the L-type Ca^{2+} channels with nifedipine prevents excessive SR Ca^{2+} loading.⁶

Furthermore, gating of L-type Ca^{2+} channels is altered in *Trdn* null myocytes.⁶ At baseline, I_{Ca} inactivation is significantly slower in *Trdn* null myocytes compared with wild-type myocytes. With β -adrenergic stimulation there is a greater increase in I_{Ca} amplitude in *Trdn* null myocytes, which is again accompanied by very slow I_{Ca} inactivation. Since preventing SR Ca^{2+} -release by blocking RyR2 channels with ryanodine or depleting the SR by incubating with thapsigargin abolishes the differences in I_{Ca} amplitude and most of the differences in inactivation, this finding was interpreted as being related to the impaired negative feedback on I_{Ca} from SR Ca^{2+} -release resulting from the structural disruption of the couplon and consequent impaired microdomain signalling. Furthermore, activation and inactivation of I_{Ca} remain significantly slower in *Trdn* null myocytes using Ba^{2+} as a charge carrier, which does not trigger SR Ca^{2+} release and does not cause Ca^{2+} -dependent inactivation. Hence, in addition to impaired SR Ca^{2+} release-induced I_{Ca} inactivation, there appears to be altered *intrinsic* I_{Ca} gating in *Trdn* null myocytes, the mechanism of which remains to be determined.⁶ On the other hand, NaCa exchanger function was not significantly changed in *Trdn* null myocytes.⁶

As described in the previous section, *Trdn* ablation impairs I_{Ca} -induced SR Ca^{2+} release despite the increased SR Ca^{2+} content by virtue of reduced RyR2s and L-type Ca^{2+} channels juxtaposition.⁶ In addition, the severe (<90%) reduction of junctin, which can desensitize RyR2 channels,¹³ may contribute to the reduction in SR Ca^{2+} release. Somewhat paradoxically, there is an increase in spontaneous SR Ca^{2+} releases in *Trdn* null myocytes upon β -adrenergic stimulation, and catecholamine-induced ventricular arrhythmias in *Trdn* null mice.⁶ It is tempting to speculate that the disruption of the couplon

architecture due to *Trdn* deletion primes the heart for ventricular arrhythmias by a number of mechanisms: First, by reduced co-expression and non-retention of Casq2 in the jSR, with reduced Casq2 protein being an established primary cause of Ca^{2+} released triggered arrhythmias even in structurally normal cardiac muscle.^{6,25} Secondly, by the drastic reduction in junctin expression.¹³ Thirdly, by causing increased Ca^{2+} influx and SR Ca^{2+} overload due to impaired I_{Ca} inactivation, leading to spontaneous Ca^{2+} release and delayed after-depolarizations with catecholaminergic surge.^{6,11,25} Fourthly, although hypothetical and not yet evaluated in the triadin model, the impaired I_{Ca} inactivation in theory may cause action potential prolongation, which is a well-established mechanism for early after-depolarizations (EADs) and EAD-mediated arrhythmias.²⁶

Interestingly, at the level of the SR, it is likely the loss of Casq2²⁵ and junctin¹³ coupled with SR Ca^{2+} overload during β -adrenergic stimulation which triggers the CPVT phenotype in the *Trdn* null mice. This is supported by observation in multiple murine models of CPVT where the common theme is reduction (100%, 60%, or modest 25%) in Casq2 with or without reduction in triadin and/or Junctin.^{11,25,27} Additionally, the loss of junctin in isolation is also shown to increase SR Ca^{2+} leak during catecholaminergic surge.¹³ It is important to note that the *direct effect* of loss of native triadin on the RyR2 complex and SR Ca^{2+} release still remains largely unknown and that experiments in *Trdn* null myocytes have not ruled out the previously held notion that loss of triadin desensitizes the RyR2 complex.⁶ It is possible that the functional consequences of loss of Casq2 and junctin dominate over the loss of triadin on SR Ca^{2+} release upon *Trdn* ablation. Future experiments that measure gating in native RyR2s incorporated in lipid bilayer or Ca^{2+} waves in permeabilized myocytes from *Trdn* null or heterozygous mice may clarify this further, although reduction of other SR Ca^{2+} -handling proteins notably Casq2 and junctin will make it difficult to gauge the effect of triadin loss in isolation.

6. Loss of function mutations in *TRDN* as a cause of CPVT in humans

CPVT is a genetic disorder characterized by stress-induced polymorphic VT and sudden death in the absence of structural heart disease.²⁸ It is primarily a paediatric disease affecting juveniles and young adults. In up to 70% of cases, mutations in the *RYR2* or *CASQ2* are the underlying culprit,²⁸ the mechanism being delayed after-depolarization-induced triggered VT during catecholamine excess.²⁸

Although *TRDN* has been an attractive candidate gene that could harbour CPVT mutations given that the *Trdn* null mouse reported in 2009 displayed a CPVT phenotype,⁶ none had been found until 2012.¹⁶ Using a candidate gene approach, mutations in the triadin (*TRDN*) and in the junctin (*ASPH*) genes were sought in a cohort of 97 CPVT patients who did not harbour *RYR2* or *CASQ2* mutations.¹⁶ No *ASPH* mutations were found; however, three mutations in *TRDN* co-segregated with the disease and displayed a recessive mode of transmission in two families.¹⁶

The proband in the first family was a 2-year-old boy who experienced syncope followed by cardiac arrest while playing with his 7-year-old brother and later died due to anoxic brain injury. His ECG after cardiac arrest showed runs of polymorphic and bidirectional VT typical of CPVT which was also displayed by the *Trdn* null

mouse.^{6,16} Molecular analysis revealed a c.del53_56ACAG homozygous deletion in exon 2 of *TRDN* which led to the deletion of four nucleotides resulting in a frame-shift of the amino acid sequence from position 18 leading to a premature stop codon at position 31. Putatively, this would lead to the deletion of the major part of the triadin protein making it either non-functional or absent.¹⁶ Three unaffected relatives who had a normal phenotype tested heterozygous for this mutation.

The proband in the second family was a 26-year-old male with recurrent episodes of syncope during exercise since infancy. Interestingly, he also displayed proximal muscle weakness suggesting the absence of triadin protein in both the cardiac and skeletal muscle which would be predicted since the same gene (*TRDN*) encodes for the skeletal and cardiac isoforms by alternative splicing.⁷ Nonetheless, the phenotype in humans consequent to a non-functional *TRDN* mutation appears to be primarily cardiac, which is consistent with the relatively preserved skeletal muscle contractile function found in *trdn* null mice.²⁹ CPVT was also diagnosed in his dizygotic twin-brother. Molecular analysis in this family revealed that the two affected patients were compound heterozygous for a c.176C>G missense mutation in exon 2 that led to the substitution of a threonine by an arginyl residue at position 59 (p.T59R) and a c.613C>T nonsense mutation in exon 8 that introduced a premature stop codon at position 205 in the amino acid sequence (p.Q205*). The latter mutation is clearly pathogenic since it would lead to either the absence of protein or the presence of a non-functional protein. Although cardiac biopsy was not performed from the probands in the two families, their mutations would predict complete absence of the triadin protein in the hearts and the skeletal muscle akin to the *Trdn* null mouse.^{6,16}

The p.T59R missense mutation in the second family proband resulted in the introduction of a positively charged arginine instead of threonine at position 59 in the transmembrane domain of the protein. Since the consequence of this mutation was unknown, it was further studied by expressing the p.T59R mutant in COS-7 cells which resulted in intracellular retention and degradation of the mutant protein.¹⁶ This was confirmed with *in vivo* expression of the mutant triadin in *Trdn* null mouse by viral transduction.¹⁶

No echocardiographic data were reported from the patients harbouring *TRDN* mutations. But if one were to extrapolate from the *Trdn* null mouse phenotype, the LV wall thickness and the LV ejection fraction may be increased.⁶ The latter was a surprising finding, since at the individual myocyte level, loss of triadin causes reduced EC coupling gain and decreased contractility,⁶ a cellular phenotype that has previously been associated with impaired contractile function of myocytes isolated from experimental models of hypertrophy and heart failure.³⁰ Clearly, more detailed studies, in particular, of the patients harbouring *TRDN* mutations, are needed to solve this puzzle.

7. Conclusions

In summary, triadin serves a crucial role in maintaining structural integrity of the cardiac CRUs and in doing so ensures functional cardiac EC coupling and tightly controlled SR Ca^{2+} release. In mice, the loss of function of *Trdn* causes a phenotype of catecholamine-induced SR Ca^{2+} overload, premature spontaneous Ca^{2+} release and Ca^{2+} -triggered polymorphic ventricular tachycardia. In humans, the loss of function mutations in the *TRDN* gene has been associated with CPVT, which is consistent with the findings from the *Trdn* null mouse model. However, the significance of the dramatic

down-regulation of triadin and junctin in failing hearts is largely unknown and needs further elucidation. Several questions remain and need more investigation, and it is crucial that they are answered for progress to occur in this field. 'Necessity is the mother of invention' and understanding the role of SR Ca^{2+} -handling proteins in health and disease is crucial for pioneering new therapeutic options for treating heart failure and ventricular arrhythmias, possibly by targeting the sub-cellular defect in SR Ca^{2+} release.

Conflict of interest: none declared.

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