REVIEWS

PHYSIOLOGY 22: 167–173, 2007; 10.1152/physiol.00005.2007

Resurgence of Cardiac T-Tubule Research

Fabien Brette[1] and Clive Orchard[2]

[1]Faculty of Life Sciences, University of Manchester, Manchester; and [2] Department of Physiology, School of Medical Sciences, University of Bristol, Bristol, United Kingdom
f.brette@manchester.ac.uk

The transverse tubules of mammalian cardiac ventricular myocytes are invaginations of the surface membrane. Recent data have revealed that their structure and function are more complex than previously believed. Here, we review current knowledge about their role in cardiac function, focusing on Ca^{2+} signaling and changes observed in pathological conditions.

Every heartbeat is triggered by the propagation of an action potential through the heart. During the cardiac action potential, Ca^{2+} enters each cardiac myocyte through L-type Ca^{2+} channels [as the Ca^{2+} current (I_{Ca})] and activates adjacent Ca^{2+} release channels [ryanodine receptors (RyR)] in the membrane of the intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR), causing Ca^{2+} release from the SR (5). Ventricular myocytes, which cause contraction of the ventricles of the heart, have invaginations of the surface membrane, called transverse (t-) tubules, which are important in ensuring rapid and synchronous contraction of the cell, and hence of the heart (13). This brief review will describe recent evidence that t-tubules are regions of the cell membrane that play a specialized role in transmembrane ion flux, and hence cell function, and that this role may change, and hence contribute to the altered function observed, during heart failure.

Structure of Cardiac T-Tubules

Mammalian cardiac myocytes differ from those of fish, amphibians, reptiles, and birds by having an extensive t-tubule system (4, 9, 34, 67). Within mammalian cardiac tissue, t-tubules occur predominantly in ventricular myocytes, being absent from pacemaking and conducting tissue and from most atrial myocytes (1, 24), although some may show a sparse distribution (e.g., Ref. 46). In ventricular myocytes, t-tubules are labile: they are absent in neonatal cells (37), and decrease when cells are kept in culture (51, 53), but little is known about the mechanisms underlying t-tubule expression and maintenance. However, in CHO cells, expression of amphisin-2, a protein that can link the plasma membrane and submembranous cytosolic scaffolds, generates narrow tubules that are continuous with the plasma membrane (49). T-tubule development also shows similarities to the development of caveolae, which requires cholesterol and caveolin-3 (21, 31, 41). Interestingly, caveolin-3-null mice show abnormalities in t-tubule organization in skeletal muscle (33), although it remains to be shown whether similar changes occur in cardiac myocytes. Cardiac t-tubule diameter is typically 100–300 nm (69), which is much larger than t-tubules in skeletal muscle [20–40 nm (32, 63)]. T-tubules are found at ~1.8-μm intervals along the long axis of the cell; roughly at every Z line. Along a Z line, t-tubules occur at regular intervals of ~1.2 μm (45) and run deep into the ventricular cell. Although most t-tubules are organized radially, they also branch and have longitudinal extensions (29, 55, 69), which can comprise up to 40% of the total t-tubule volume (69). Thus t-tubules form a complex network of branching tubules with both transverse and longitudinal elements rather than a simple transverse system of tubules, as its name might suggest. Alternative names have therefore been proposed, such as the transverse-axial tubular system (TATS), sarcolemmal Z rete, or sarcolemmal tubule network (4, 29, 69), although t-tubules remains the standard term. This network of membrane within the cell conducts the cardiac action potential into the center of the myocyte.

T-Tubules and Electrical Activity

Physical and functional distribution of ion channels and transporters

The function of t-tubules depends not only on their structure but also on the proteins within and adjacent to the t-tubule membrane. Immunocytochemistry in conjunction with confocal microscopy has been widely used to investigate the location of proteins within cardiac ventricular myocytes. Many important proteins are present in the t-tubule membrane, including ion channels such as L-type Ca^{2+} channels (LTCC), Na^+ channels, and K^+ channels; and transporters such as Na^+/Ca^{2+} exchanger (NCX) and Na^+/K^+ ATPase. Key proteins involved in the modulation of cardiac function are also present at the t-tubules, including nitric oxide synthase type 3 and cAMP-dependent pathway proteins, such as the β-adrenoceptor, G_s, adenylate cyclase, and AKAP (see Ref. 13 for review). In addition, LTCCs are highly co-localized with RyRs at the dyad, where the sarcolemmal and junctional SR membranes are closely apposed (20, 65); dyads are more abundant at the t-tubules than at the surface membrane, e.g., 25% at the cell surface compared with 75% at the t-tubules in rat (18). Moreover, the SR Ca^{2+} ATPase (SERCA2) and its regulatory protein phospholamban are located throughout the SR membrane including at the Z

line, adjacent to the t-tubule (16, 54), although the location of SERCA2 relative to the dyad is not yet known.

Although immunocytochemistry has been useful to localize proteins in cardiac myocytes, protein distribution is not necessarily reflected in protein function, which also depends on local environment and regulation and accessory proteins (13). Furthermore, quantification of protein densities from immunocytochemistry is difficult and may depend on the antibodies used. To quantify protein function in the t-tubules, a different approach is required. The first studies to show that membrane currents are located predominantly in the t-tubules used the fact that such channels are in a restricted diffusion space. Thus, when the composition of the solution bathing a cell was changed rapidly, some currents, for example I_{Ca}, showed an initial rapid change followed by a slower change. The first phase represented about 36% of I_{Ca}, the slow phase 64%, suggesting that these fractions of I_{Ca} are located in the surface and t-tubule membranes, respectively (66). More recently, a different approach has been developed: the detubulation technique. This is an adaptation of the "osmotic shock" technique used previously to detubulate skeletal muscle (e.g., Ref. 43). The osmotic shock and consequent changes of cell volume, caused by application and removal of a membrane permeant agent (formamide), result in the t-tubules being physically and functionally uncoupled from the surface membrane (44). Importantly, this procedure has no effect on the function of rat atrial myocytes, which lack t-tubules (11), which strongly argues against a direct effect of formamide. Detubulated rat ventricular myocytes show a decrease in cell capacitance (which reflects membrane area) of ~30% (14–18, 44, 47, 75), a value close to the estimate of membrane present within the t-tubules obtained by electron microscopy (56). This technique enables functional mapping of currents mediated by ion channels and transporters. Using detubulation, we have shown that 80% of I_{Ca} (17) and 63% of I_{NCX} (25) are present in the t-tubules of rat ventricular myocytes. Thus the major Ca^{2+} flux pathways are more concentrated in the t-tubules than in the surface membrane (Table 1). Some Na^+ handling proteins are also concentrated in the t-tubules, e.g., Na^+-K^+-ATPase and the neuronal isoform of the Na^+ current (14, 15, 25). In contrast, K^+ currents are evenly distributed between the surface and t-tubule membranes, except for I_{SS} which is concentrated at the t-tubules (47). Interestingly, we have found only one ionic current, which is more concentrated at the surface membrane than the t-tubules: the cardiac isoform of Na^+ current (14, 15). This technique has been adopted by other laboratories, which have shown similar results for the distribution of NCX (72) and have revealed the isoform distribution of Na^+-K^+-ATPase (3).

Table 1. Subcellular distribution of key currents in rat ventricular myocyte

	% in T-Tubules	Density (T-Tubules/ext SL)	Reference
I_{Ca}	80	6	17
I_{NaCa}	63	3	25
I_{NaC}	22	0.6	15
I_{NaN}	80	9	15
I_{NaK}	59	3	25
I_{TO}	29	1.5	47
I_{SS}	71	9	47
I_{K}	27	1.3	47
I_{K1}	26	1.2	47

T-tubules represent typically ~30% of the cell membrane. ext SL, external sarcolemma; I_{Ca}, calcium current; I_{NCX}, Na^+/Ca^{2+} exchange current; I_{NaC}, cardiac sodium current; I_{NaN}, neuronal sodium current; I_{NaK}, Na-K ATPase current; I_{TO}, transient outward current; I_{SS}, sustained potassium current; I_{K}, delayed rectifier current; I_{K1}, inward rectifier current. K^+ current values are calculated from Ref. 47.

Electrical properties of t-tubules

The t-tubules provide an electrical pathway from the surface of a cardiac myocyte to the cell interior. In addition to this structural role, many ion channels and transporters are concentrated within the t-tubules (above). Thus it seems likely that the electrical activity of the t-tubules differs from the surface membrane. However, because the t-tubules are long narrow invaginations, it has long been uncertain whether the voltage clamp technique, used to measure membrane currents, adequately controls the voltage across the t-tubule membrane. We have explored the quality of voltage control by investigating Na^+ current kinetics in control and detubulated myocytes. This current is large and fast and, therefore, prone to voltage escape, which changes its kinetics. We found that Na^+ current showed similar activation and inactivation kinetics over a wide range of test voltages in control and detubulated myocytes (14). In addition, these values were independent of the series resistance in the range used [$\leq$4.2 MΩ before compensation (14)]. These results clearly suggest that the t-tubules can be effectively voltage clamped. This is consistent with previous work in guinea pig ventricular myocytes suggesting that t-tubules have little effect on the quality of voltage clamp (68).

Investigation of the electrical properties of the t-tubules is also hampered by our inability to record the electrical activity of the t-tubule membrane only. We have, however, investigated the characteristics of the action potential in control (t-tubules and surface membrane) and detubulated (surface membrane only) myocytes to obtain an indirect estimate of the electrical activity of the t-tubules. The main effect of loss of t-tubules was to decrease action potential duration, whereas action potential amplitude and resting membrane potential were unchanged (18). It is likely that the decrease in action potential duration is due to loss of I_{Ca} and I_{NCX}, which carry positive charge into the cell and are concentrated at the t-tubules, with a limited role for K^+ currents, which are evenly distrib-

uted (Table 1). Calculations from these data suggest that the t-tubule action potential should be ~5.5 longer than at the cell surface. This challenging speculation of course requires experimental confirmation when direct recording from t-tubule membrane becomes possible. However, in a normal myocyte, the action potential will be uniform throughout the cell membrane (the mean of that generated by the two membranes) because of the tight electrical coupling of the surface and t-tubule membranes. This has been described recently using mathematical modeling (57); indeed, the action potential is delayed by only 2–3 ms in the t-tubules, as previously described in skeletal myocytes (42).

T-Tubules and Calcium Cycling

It was assumed for many years that t-tubules were simple invaginations of the surface membrane of cardiac ventricular myocytes, which allowed propagation of the action potential to the cell interior, allowing spatially and temporally synchronous Ca^{2+} release throughout the cell. This idea received support with the development of Ca^{2+}-sensitive dyes and fast imaging techniques, which showed that cells lacking t-tubules, for example those from the atria and Purkinje fibers (8, 24, 40), cultured ventricular cells (51), and cells from the ventricles of newborn animals (37), showed asynchronous Ca^{2+} release, with Ca^{2+} rising initially at the surface membrane, in contrast to ventricular myocytes that showed synchronous Ca^{2+} release (11, 16, 37, 75). However, it now appears that t-tubules are not simple invaginations; many of the key proteins involved in excitation-contraction coupling are located predominantly at the t-tubules (Table 1), which suggests that they play a specialized and important role in Ca^{2+} handling and excitation-contraction coupling.

The T-Tubules Represent a Specialized Region for Transmembrane Ion Flux

The detubulation technique has allowed quantification of the distribution of membrane currents between the t-tubule and surface membranes (above). It is striking that the currents involved in Ca^{2+} handling appear to be located predominantly within the t-tubules (Table 1). This is interesting for a number of reasons, not least because it places them within a domain with restricted diffusion access to the bulk extracellular space. An intracellular restricted diffusion space appears to exist under the sarcolemma ["fuzzy space" (48)] so that the concentration of ions adjacent to the sarcolemma, and thus to the intracellular face of ion flux pathways, may be different from that in the bulk intracellular space (4). It now seems feasible that concentration of ion flux pathways in the t-tubules, coupled to restricted diffusion, may result in these proteins also being exposed to an extracellular ion concentration that is different from that in the bulk extracellular solution. Computer modelling suggests that this may result in Ca^{2+} depletion in the t-tubule lumen during activity, which may limit Ca^{2+} influx (57). Similarly, a recent study has shown slow K^+ diffusion in the t-tubules (~85 $\mu m^2/s$), which can lead to K^+ accumulation (71). This might be particularly important in modulating cell function in small rodents (mouse and rat), in which repolarization is due predominantly to the large transient outward current.

"Immunocytochemistry has shown that many key components of major signalling pathways are located predominantly at the t-tubules . . ."

Ca^{2+} Influx and Ca^{2+} Release at the T-Tubules

Ca^{2+} influx via the LTCCs triggers Ca^{2+} release from the SR; it is predominantly this Ca^{2+} that activates the contractile proteins and causes contraction of the cardiac ventricular myocyte. I_{Ca} also helps to load the SR with Ca^{2+} ready for subsequent release (27) and thus plays a pivotal role in cardiac cell function.

Comparison of I_{Ca} in intact ventricular myocytes, in which it flows predominantly across the t-tubule membrane, with that in detubulated myocytes, in which it flows across the surface membrane, suggests that the current is different at the two sites. Detubulation causes not just a marked decrease in current amplitude, consistent with loss of channels (above), but the remaining current exhibits a slow time course of inactivation (17). This implies that t-tubular I_{Ca} is large but inactivates quickly, whereas that at the surface membrane is small but inactivates slowly. The rapid inactivation of t-tubular I_{Ca} does not appear to be due to depletion of Ca^{2+} within the t-tubule lumen or to a difference in voltage-dependent inactivation, because inactivation of I_{Ca} is the same in control and detubulated myocytes when Ba^{2+} is used as the charge carrier (17). However inhibition of SR Ca^{2+} release abolishes the difference in inactivation rate, suggesting that inactivation of I_{Ca} by Ca^{2+} released from the SR is more profound at the t-tubules than at the surface membrane. Although the mechanism of this differential Ca^{2+}-dependent inactivation is unknown, it does not appear to be due to differences in SR Ca^{2+} release at the two sites (below).

The difference in the kinetics of I_{Ca} at the t-tubules and surface membrane is important, because it means that the amount of Ca^{2+} entering the cell via I_{Ca} at the surface membrane is larger than might be expected from its amplitude. Since the inactivation phase of I_{Ca} appears to

be important in loading the SR with Ca^{2+}, this suggests that surface I_{Ca} may be "specialized" for this role, an idea supported by the observation that, although detubulation slows the rate of SR loading, the slowing is not as great as might be expected from the decrease in current amplitude (10). Conversely, a large, rapidly inactivating I_{Ca}, as in the t-tubules, has the characteristics required for an effective trigger for Ca^{2+} release, which depends more of the early phase of I_{Ca} (27, 60).

Although the characteristics of I_{Ca} appear to be different at the t-tubule and surface membranes, the coupling of I_{Ca} to SR Ca^{2+} release is surprisingly consistent: from the amplitude of I_{Ca} in control and detubulated myocytes, and using reasonable assumptions about single channel current, it is possible to calculate the number of Ca channels in the t-tubule and surface membranes of a rat ventricular myocyte (143,400 and 48,200, respectively). Using electron microscopy data to calculate the number of RyR clusters (Ca^{2+} release sites or junctions) at the t-tubules and cell surface, it is thus possible to calculate the number of Ca^{2+} channels per junction: ~35 at the two sites (18) (FIGURE 1). This is borne out in measurements of the amount of Ca^{2+} released for a given Ca^{2+} influx at the two sites, which is not significantly different (17).

Thus I_{Ca} amplitude and hence Ca^{2+} release are smaller at the cell surface because of fewer Ca^{2+} channels. However, the number of Ca^{2+} channels per junction and the Ca^{2+} release for a given Ca^{2+} influx is the same at the two sites, although the released Ca^{2+} appears to inactivate I_{Ca} more effectively at the t-tubules than at the surface membrane.

Local regulation of Ca^{2+} flux at the t-tubules

Local regulation of membrane proteins is known to occur in cardiac myocytes. β_2-Adrenergic stimulation, for example, stimulates I_{Ca} without affecting other proteins, apparently because phosphodiesterases spatially limit the effect of locally produced cAMP (28). Caveolae [50- to 80-nm diameter invaginations of the surface and t-tubule membranes (4)] have also been implicated in compartmentalized signaling in cardiac myocytes (see Ref. 23 for review). Immunocytochemistry has shown that many key components of major signalling pathways are located predominantly at the t-tubules, and some proteins, such as RyR, form macromolecular complexes that include kinases and phosphatases whose action is therefore localized (6, 74). It has also been shown that I_{Ca} in the t-tubules is inactivated more rapidly by Ca^{2+} released from the SR, compared with I_{Ca} at the surface membrane, even though the amount of Ca^{2+} released by a given Ca^{2+} influx is the same at the two sites (above). This inactivation depends on calmodulin, which is prebound to the channel (26, 76); the data suggest, therefore, that this Ca^{2+} activated pathway is more active at the t-tubules than the surface membrane, although the reason is unknown. Similarly, it has been shown that the fractional increase of I_{Ca} produced by the adrenergic agonist isoprenaline is greater in control than in

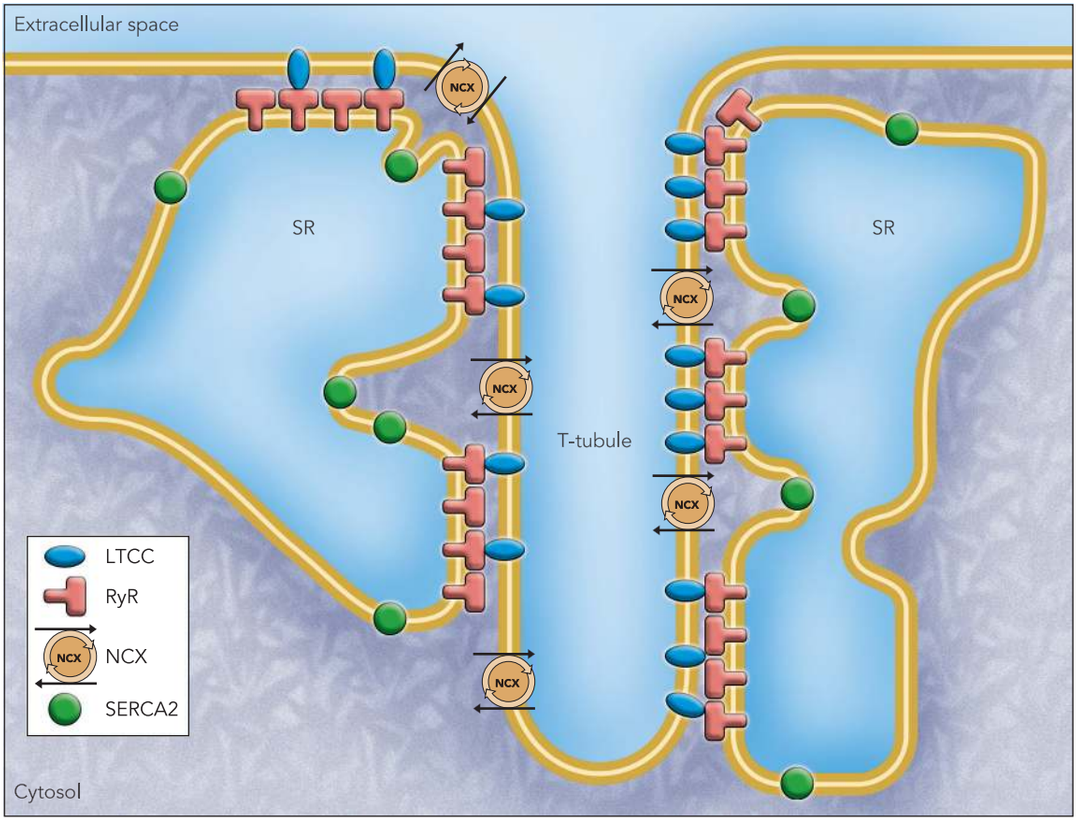

FIGURE 1. **Proteins involved in calcium signaling in mammalian ventricular myocytes**
Junctional sarcoplasmic reticulum (SR) membrane in close proximity to the sarcolemma forms dyads, which are present mainly in the transverse tubules (TT) but also at the surface membrane. Dyads comprise a cluster of ryanodine receptors (RyRs) adjacent to, and under the functional control of, L-type Ca^{2+} channels; this forms a Ca^{2+} release unit. Proteins involved in Ca^{2+} re-uptake (SR Ca^{2+}-ATPase, SERCA2) and extrusion [Na^+/Ca^{2+} exchanger (NCX)] are preferentially located within or near the transverse tubules.

detubulated myocytes, suggesting that I_{Ca} is better coupled to the adrenergic pathway at the t-tubules than at the surface membrane (16). In contrast, isoprenaline-induced phosphorylation of the regulatory protein phospholamban, which is found in the SR membrane throughout the cell, is unaffected by detubulation (16).

These data provide evidence that ion flux pathways are differentially regulated at the t-tubules and surface membrane; as a consequence, their function may be different at the two sites, particularly in the presence of tonic activation of signalling pathways.

T-Tubules and Pathophysiology

Surprisingly few studies have examined t-tubule structure in living cells from heart failure models or patients. In a canine model of tachycardia-induced heart failure, a marked loss of t-tubules in ventricular myocytes has been described (2, 39). Loss of t-tubules has also been observed in a rabbit model of heart failure (t-tubule area 59% of sham) (59). In human failing myocytes, the issue of whether t-tubules are altered is starting to be addressed. Although a prominent t-tubule network has been reported in failing human ventricular myocytes, this study was conducted without control myocytes (51). Another recent study reported an increase in the size of the t-tubules and more longitudinal extensions than in control human heart (19).

Although loss of t-tubules cannot be responsible for all the changes of Ca^{2+} handling that occur during heart failure, it is notable that detubulation results in many of the functional changes observed in heart failure, suggesting that loss of t-tubules in heart failure may contribute to and exacerbate the phenotype observed in failure. These changes include a reduced and slowed Ca^{2+} transient, less synchronized SR Ca^{2+} release, a negative force-frequency relationship, a decreased response of I_{Ca} to β-adrenergic stimulation, a decreased contractile response to cardiac glycosides, and decreased frequency-dependent facilitation of I_{Ca} (10, 16, 17, 30).

However, it remains unclear how t-tubule disorganization influences excitation-contraction coupling during heart failure. Several recent studies have correlated t-tubule remodelling and desynchronization of SR Ca^{2+} release (19, 51, 52, 70). Instead of marked t-tubule loss (above), two recent studies showed subtle reorganization of the t-tubular network (52, 70) that, in these animal models of heart failure (mouse and rat), was directly linked to changes in the synchronicity Ca^{2+} release. Changes in t-tubule organization may alter the spatial distribution of LTCCs (which are concentrated at the t-tubules, above), whereas RyR distribution remains regular, resulting in some orphaned RyRs (70). Orphaned RyRs (70) and/or an increased number of longitudinal t-tubules (52) are likely to induce a delay in SR Ca^{2+} release because of the increased average distance between LTCCs and RyRs, thereby causing desynchronization of SR Ca^{2+} release (51, 52, 70). The same subtle t-tubule reorganization has been observed in failing human heart (19), and computer modelling has shown that this can reduce the synchrony of Ca^{2+} spark production and lead to the appearance of late Ca^{2+} sparks and greater nonuniformity of intracellular Ca^{2+} (19), as observed in animal models of heart failure (50, 58) (FIGURE 2). These data suggest that geometric factors can play an important role in the pathophysiology of human heart failure. This is consistent with the observation of decreased excitation-contraction coupling gain (i.e., the ability of a given I_{Ca} to trigger SR Ca^{2+} release) in a rat model of heart failure in which it was speculated that altered excitation-contraction coupling was due to t-tubule remodelling (35, 36).

Whether changes in t-tubule organization are responsible for desynchronization of SR Ca^{2+} release during heart failure is still under debate (7): altered I_{Ca} characteristics can cause desynchronization of SR Ca^{2+} release in a cat model of heart failure (38) in the absence of changes in t-tubule structure, probably because of reduced Ca^{2+} influx due in part to alterations in early repolarization of the action potential (38). This phase is critical to SR Ca^{2+} release (61, 62) and is altered in most animal models and human heart failure (see Ref. 73 for review). However, in the majority of studies in animal models of heart failure, I_{Ca} amplitude (expressed as current density) is unchanged (for review, see Ref. 12). Thus the ability of I_{Ca} to trigger SR Ca^{2+} release might be altered due to a change in action potential shape and not a decrease in current density. At least two mechanisms might therefore participate in desynchronization of SR Ca^{2+} release during heart failure: t-tubule remodelling and a change in the early phase of the action potential.

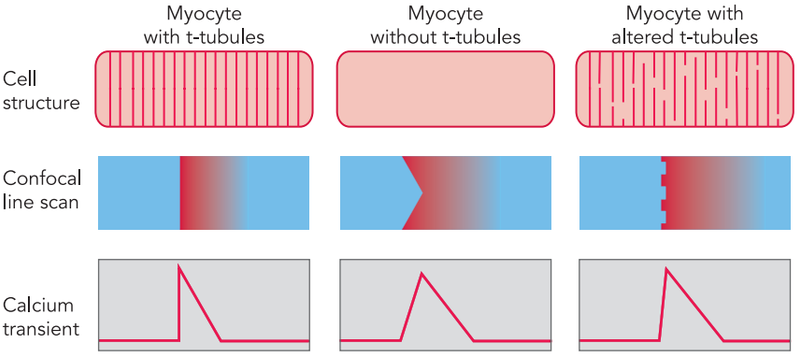

FIGURE 2. **T-tubules and calcium signaling in mammalian ventricular myocytes**
Each panel shows a cartoon of the cell structure (*top*), a confocal linescan image of intracellular Ca^{2+} (*middle*), and the Ca^{2+} transient (*bottom*). In a normal ventricular myocyte (*left*), there is an extensive t-tubule network that ensures synchronization of calcium release. In a myocyte without t-tubules (i.e., detubulated, atrial, neonatal; *middle*), Ca^{2+} release is initiated at the edge of the cell and then propagates toward the center. In a myocyte where there is a subtle reorganization of the t-tubules (e.g., some animal models and human myocytes from failing hearts; *right*), desynchronization of the Ca^{2+} transient is observed.

Further studies are needed to clarify the relative contribution of these mechanisms. The unchanged I_{Ca} density is interesting because this suggest that the cardiac myocyte, despite hypertrophy (cell size nearly doubles during heart failure), is able to maintain constant Ca^{2+} entry. The decrease in t-tubule density without a concomitant decrease in I_{Ca} density seems paradoxical, since LTCC are concentrated in the t-tubules (above). However, recent work has shown that, although the density of LTCC decreases [assessed by measurement of gating charge movement (39)], I_{Ca} density is maintained by increased phosphorylation, and hence activity, of the channel (22, 64). It also remains possible that the distribution of LTCCs changes so that they are no longer preferentially localized in the t-tubules during heart failure.

Conclusions and Future Directions

The last few years have seen an enormous increase in our knowledge and understanding of cardiac t-tubules. However, it is frustrating that there is still no method that allows direct recording from the t-tubule membrane, although voltage-sensitive dyes and the ability to isolate a single t-tubule using scanning ion conductance microscopy hold promise for the future. Until then, our knowledge is indirect: we still have, for example, no direct measurements of currents flowing across the t-tubule membrane or knowledge of the environment in which the ion flux proteins are located, for example, the composition of the t-tubule membrane. More fundamentally, it is still not clear how t-tubules, which appear to be extremely labile, are formed and maintained, or how ion flux pathways are localized to the t-tubule membrane, or how t-tubule structure, composition, and function change during cardiac pathology. Until we are able to make such measurements and answer these questions, our knowledge of the importance of the t-tubules, so tantilizingly revealed in the last few years, will remain hazy. ■

We thank Dr. Laurent Sallé for making a preliminary version of FIGURE 1.

Work in our laboratories is supported by the Wellcome Trust and British Heart Foundation. F. Brette is a Wellcome Trust Research Fellow.

References

1. Ayettey AS, Navaratnam V. The T-tubule system in the specialized and general myocardium of the rat. *J Anat* 127: 125–140, 1978.
2. Balijepalli RC, Lokuta AJ, Maertz NA, Buck JM, Haworth RA, Valdivia HH, Kamp TJ. Depletion of t-tubules and specific subcellular changes in sarcolemmal proteins in tachycardia-induced heart failure. *Cardiovasc Res* 59: 67–77, 2003.
3. Berry RG, Despa S, Fuller W, Bers DM, Shattock MJ. Differential distribution and regulation of mouse cardiac Na^+/K^+-ATPase alpha1 and alpha2 subunits in T-tubule and surface sarcolemmal membranes. *Cardiovasc Res* 73: 92–100, 2007.
4. Bers DM. *Excitation-Contraction Coupling and Cardiac Contractile Force* (2nd ed.). Dordrecht, Netherlands: Kluwer Academic, 2001.
5. Bers DM. Cardiac excitation-contraction coupling. *Nature* 415: 198–205, 2002.
6. Bers DM. Macromolecular complexes regulating cardiac ryanodine receptor function. *J Mol Cell Cardiol* 37: 417–429, 2004.
7. Bers DM. Altered cardiac myocyte Ca regulation in heart failure. *Physiology Bethesda* 21: 380–387, 2006.
8. Bootman MD, Higazi DR, Coombes S, Roderick HL. Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes. *J Cell Sci* 119: 3915–3925, 2006.
9. Bossen EH, Sommer JR, Waugh RA. Comparative stereology of the mouse and finch left ventricle. *Tissue Cell* 10: 773–784, 1978.
10. Brette F, Despa S, Bers DM, Orchard CH. Spatiotemporal characteristics of SR Ca^{2+} uptake and release in detubulated rat ventricular myocytes. *J Mol Cell Cardiol* 39: 804–812, 2005.
11. Brette F, Komukai K, Orchard CH. Validation of formamide as a detubulation agent in isolated rat cardiac cells. *Am J Physiol Heart Circ Physiol* 283: H1720–H1728, 2002.
12. Brette F, Leroy J, Le Guennec JY, Salle L. Ca^{2+} currents in cardiac myocytes: Old story, new insights. *Prog Biophys Mol Biol* 91: 1–82, 2006.
13. Brette F, Orchard C. T-tubule function in mammalian cardiac myocytes. *Circ Res* 92: 1182–1192, 2003.
14. Brette F, Orchard CH. Density and sub-cellular distribution of cardiac and neuronal sodium channel isoforms in rat ventricular myocytes. *Biochem Biophys Res Commun* 348: 1163–1166, 2006.
15. Brette F, Orchard CH. No apparent requirement for neuronal sodium channels in excitation-contraction coupling in rat ventricular myocytes. *Circ Res* 98: 667–674, 2006.
16. Brette F, Rodriguez P, Komukai K, Colyer J, Orchard CH. Beta-adrenergic stimulation restores the Ca transient of ventricular myocytes lacking t-tubules. *J Mol Cell Cardiol* 36: 265–275, 2004.
17. Brette F, Salle L, Orchard CH. Differential modulation of L-type Ca^{2+} current by SR Ca^{2+} release at the T-tubules and surface membrane of rat ventricular myocytes. *Circ Res* 95: e1–e7, 2004.
18. Brette F, Salle L, Orchard CH. Quantification of calcium entry at the T-tubules and surface membrane in rat ventricular myocytes. *Biophys J* 90: 381–389, 2006.
19. Cannell MB, Crossman DJ, Soeller C. Effect of changes in action potential spike configuration, junctional sarcoplasmic reticulum micro-architecture and altered t-tubule structure in human heart failure. *J Muscle Res Cell Motil* 27: 297–306, 2006.
20. Carl SL, Felix K, Caswell AH, Brandt NR, Ball WJ Jr, Vaghy PL, Meissner G, Ferguson DG. Immunolocalization of sarcolemmal dihydropyridine receptor and sarcoplasmic reticular triadin and ryanodine receptor in rabbit ventricle and atrium. *J Cell Biol* 129: 672–682, 1995.
21. Carozzi AJ, Ikonen E, Lindsay MR, Parton RG. Role of cholesterol in developing T-tubules: analogous mechanisms for T-tubule and caveolae biogenesis. *Traffic* 1: 326–341, 2000.
22. Chen X, Piacentino V, III, Furukawa S, Goldman B, Margulies KB, Houser SR. L-type Ca^{2+} channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. *Circ Res* 91: 517–524, 2002.
23. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev* 84: 1341–1379, 2004.
24. Cordeiro JM, Spitzer KW, Giles WR, Ershler PE, Cannell MB, Bridge JH. Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. *J Physiol* 531: 301–314, 2001.
25. Despa S, Brette F, Orchard CH, Bers DM. Na/Ca exchange and Na/K-ATPase function are equally concentrated in transverse tubules of rat ventricular myocytes. *Biophys J* 85: 3388–3396, 2003.

26. Erickson MG, Alseikhan BA, Peterson BZ, Yue DT. Preassociation of calmodulin with voltage-gated Ca^{2+} channels revealed by FRET in single living cells. *Neuron* 31: 973–985, 2001.

27. Fabiato A. Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *J Gen Physiol* 85: 291–320, 1985.

28. Fischmeister R, Castro LR, bi-Gerges A, Rochais F, Jurevicius J, Leroy J, Vandecasteele G. Compartmentation of cyclic nucleotide signaling in the heart: the role of cyclic nucleotide phosphodiesterases. *Circ Res* 99: 816–828, 2006.

29. Forbes MS, Hawkey LA, Sperelakis N. The transverse-axial tubular system (TATS) of mouse myocardium: its morphology in the developing and adult animal. *Am J Anat* 170: 143–162, 1984.

30. Fowler MR, Dobson RS, Orchard CH, Harrison SM. Functional consequences of detubulation of isolated rat ventricular myocytes. *Cardiovasc Res* 62: 529–537, 2004.

31. Franzini-Armstrong C. Simultaneous maturation of transverse tubules and sarcoplasmic reticulum during muscle differentiation in the mouse. *Dev Biol* 146: 353–363, 1991.

32. Franzini-Armstrong C, Landmesser L, Pilar G. Size and shape of transverse tubule openings in frog twitch muscle fibers. *J Cell Biol* 64: 493–497, 1975.

33. Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, Hou H Jr, Kneitz B, Edelmann W, Lisanti MP. Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and t-tubule abnormalities. *J Biol Chem* 276: 21425–21433, 2001.

34. Galli GL, Taylor EW, Shiels HA. Calcium flux in turtle ventricular myocytes. *Am J Physiol Regul Integr Comp Physiol* 291: R1781–R1789, 2006.

35. Gomez AM, Guatimosim S, Dilly KW, Vassort G, Lederer WJ. Heart failure after myocardial infarction: altered excitation-contraction coupling. *Circulation* 104: 688–693, 2001.

36. Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* 276: 800–806, 1997.

37. Haddock PS, Coetzee WA, Cho E, Porter L, Katoh H, Bers DM, Jafri MS, Artman M. Subcellular $[Ca^{2+}]_i$ gradients during excitation-contraction coupling in newborn rabbit ventricular myocytes. *Circ Res* 85: 415–427, 1999.

38. Harris DM, Mills GD, Chen X, Kubo H, Berretta RM, Votaw VS, Santana LF, Houser SR. Alterations in early action potential repolarization causes localized failure of sarcoplasmic reticulum Ca^{2+} release. *Circ Res* 96: 543–550, 2005.

39. He J, Conklin MW, Foell JD, Wolff MR, Haworth RA, Coronado R, Kamp TJ. Reduction in density of transverse tubules and L-type Ca^{2+} channels in canine tachycardia-induced heart failure. *Cardiovasc Res* 49: 298–307, 2001.

40. Huser J, Lipsius SL, Blatter LA. Calcium gradients during excitation-contraction coupling in cat atrial myocytes. *J Physiol* 494: 641–651, 1996.

41. Ishikawa H. Formation of elaborate networks of T-system tubules in cultured skeletal muscle with special reference to the T-system formation. *J Cell Biol* 38: 51–66, 1968.

42. Jack JJB, Noble D, Tsien RW. *Electric Current Flow in Excitable Cells*. Oxford, UK: Oxford Univ. Press, 1985.

43. Jaimovich E, Venosa RA, Shrager P, Horowicz P. Density and distribution of tetrodotoxin receptors in normal and detubulated frog sartorius muscle. *J Gen Physiol* 67: 399–416, 1976.

44. Kawai M, Hussain M, Orchard CH. Excitation-contraction coupling in rat ventricular myocytes after formamide-induced detubulation. *Am J Physiol Heart Circ Physiol* 277: H603–H609, 1999.

45. Kieval RS, Bloch RJ, Lindenmayer GE, Ambesi A, Lederer WJ. Immunofluorescence localization of the Na-Ca exchanger in heart cells. *Am J Physiol Cell Physiol* 263: C545–C550, 1992.

46. Kirk MM, Izu LT, Chen-Izu Y, McCulle SL, Wier WG, Balke CW, Shorofsky SR. Role of the transverse-axial tubule system in generating calcium sparks and calcium transients in rat atrial myocytes. *J Physiol* 547: 441–451, 2003.

47. Komukai K, Brette F, Yamanushi TT, Orchard CH. K^+ current distribution in rat sub-epicardial ventricular myocytes. *Pflügers Arch* 444: 532–538, 2002.

48. Lederer WJ, Niggli E, Hadley RW. Sodium-calcium exchange in excitable cells: fuzzy space. *Science* 248: 283, 1990.

49. Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, Ochoa GC, Farsad K, Wenk MR, De Camilli P. Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. *Science* 297: 1193–1196, 2002.

50. Litwin SE, Zhang D, Bridge JH. Dyssynchronous Ca(2+) sparks in myocytes from infarcted hearts. *Circ Res* 87: 1040–1047, 2000.

51. Louch WE, Bito V, Heinzel FR, Macianskiene R, Vanhaecke J, Flameng W, Mubagwa K, Sipido KR. Reduced synchrony of Ca^{2+} release with loss of T-tubules-a comparison to Ca^{2+} release in human failing cardiomyocytes. *Cardiovasc Res* 62: 63–73, 2004.

52. Louch WE, Mork HK, Sexton J, Stromme TA, Laake P, Sjaastad I, Sejersted OM. T-tubule disorganization and reduced synchrony of Ca^{2+} release in murine cardiomyocytes following myocardial infarction. *J Physiol* 574: 519–533, 2006.

53. Mitcheson JS, Hancox JC, Levi AJ. Action potentials, ion channel currents and transverse tubule density in adult rabbit ventricular myocytes maintained for 6 days in cell culture. *Pflügers Arch* 431: 814–827, 1996.

54. Musa H, Lei M, Honjo H, Jones SA, Dobrzynski H, Lancaster MK, Takagishi Y, Henderson Z, Kodama I, Boyett MR. Heterogeneous expression of Ca(2+) handling proteins in rabbit sinoatrial node. *J Histochem Cytochem* 50: 311–324, 2002.

55. Ogata T, Yamasaki Y. High-resolution scanning electron microscopic studies on the three-dimensional structure of the transverse-axial tubular system, sarcoplasmic reticulum and intercalated disc of the rat myocardium. *Anat Rec* 228: 277–287, 1990.

56. Page E. Quantitative ultrastructural analysis in cardiac membrane physiology. *Am J Physiol Cell Physiol* 235: C147–C158, 1978.

57. Pasek M, Simurda J, Christe G. The functional role of cardiac T-tubules explored in a model of rat ventricular myocytes. *Philos Transact A Math Phys Eng Sci* 364: 1187–1206, 2006.

58. Piacentino V, III, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, Houser SR. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res* 92: 651–658, 2003.

59. Quinn FR, Currie S, Duncan AM, Miller S, Sayeed R, Cobbe SM, Smith GL. Myocardial infarction causes increased expression but decreased activity of the myocardial Na^+-Ca^{2+} exchanger in the rabbit. *J Physiol* 553: 229–242, 2003.

60. Rose WC, Balke CW, Wier WG, Marban E. Macroscopic and unitary properties of physiological ion flux through L-type Ca^{2+} channels in guinea-pig heart cells. *J Physiol* 456: 267–284, 1992.

61. Sah R, Ramirez RJ, Backx PH. Modulation of Ca(2+) release in cardiac myocytes by changes in repolarization rate: role of phase-1 action potential repolarization in excitation-contraction coupling. *Circ Res* 90: 165–173, 2002.

62. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, Zobel C, Backx PH. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I(to)). *J Physiol* 546: 5–18, 2003.

63. Sandow A. Excitation-contraction coupling in skeletal muscle. *Pharmacol Rev* 17: 265–320, 1965.

64. Schroder F, Handrock R, Beuckelmann DJ, Hirt S, Hullin R, Priebe L, Schwinger RHG, Weil J, Herzig S. Increased availability and open probability of single L-type calcium channels from failing compared with nonfailing human ventricle. *Circulation* 98: 969–976, 1998.

65. Scriven DR, Dan P, Moore ED. Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophys J* 79: 2682–2691, 2000.

66. Shepherd N, McDonough HB. Ionic diffusion in transverse tubules of cardiac ventricular myocytes. *Am J Physiol Heart Circ Physiol* 275: H852–H860, 1998.

67. Shiels HA, White E. Temporal and spatial properties of cellular Ca^{2+} flux in trout ventricular myocytes. *Am J Physiol Regul Integr Comp Physiol* 288: R1756–R1766, 2005.

68. Shirokov R, Levis R, Shirokova N, Rios E. Two classes of gating current from L-type Ca channels in guinea pig ventricular myocytes. *J Gen Physiol* 99: 863–895, 1992.

69. Soeller C, Cannell MB. Examination of the transverse tubular system in living cardiac rat myocytes by 2-photon microscopy and digital image-processing techniques. *Circ Res* 84: 266–275, 1999.

70. Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci USA* 103: 4305–4310, 2006.

71. Swift F, Stromme TA, Amundsen B, Sejersted OM, Sjaastad I. Slow diffusion of K^+ in the T tubules of rat cardiomyocytes. *J Appl Physiol* 101: 1170–1176, 2006.

72. Thomas MJ, Sjaastad I, Andersen K, Helm PJ, Wasserstrom JA, Sejersted OM, Ottersen OP. Localization and function of the Na^+/Ca^{2+}-exchanger in normal and detubulated rat cardiomyocytes. *J Mol Cell Cardiol* 35: 1325–1337, 2003.

73. Tomaselli GF, Marban E. Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res* 42: 270–283, 1999.

74. Wehrens XH, Lehnart SE, Marks AR. Intracellular calcium release and cardiac disease. *Annu Rev Physiol* 67: 69–98, 2005.

75. Yang Z, Pascarel C, Steele DS, Komukai K, Brette F, Orchard CH. Na^+-Ca^{2+} exchange activity is localized in the T-tubules of rat ventricular myocytes. *Circ Res* 91: 315–322, 2002.

76. Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature* 399: 159–162, 1999.