T-Tubule Function in Mammalian Cardiac Myocytes

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Abstract—The transverse tubules (t-tubules) of mammalian cardiac ventricular myocytes are invaginations of the surface membrane. Recent studies have suggested that the structure and function of the t-tubules are more complex than previously believed; in particular, many of the proteins involved in cellular Ca\(^{2+}\) cycling appear to be concentrated at the t-tubule. Thus, the t-tubules are an important determinant of cardiac cell function, especially as the main site of excitation-contraction coupling, ensuring spatially and temporally synchronous Ca\(^{2+}\) release throughout the cell. Changes in t-tubule structure and protein expression occur during development and in heart failure, so that changes in the t-tubules may contribute to the functional changes observed in these conditions. The purpose of this review is to provide an overview of recent studies of t-tubule structure and function in cardiac myocytes. (Circ Res. 2003;92:1182-1192.)

Key Words: cardiac muscle • t-tubules • excitation-contraction coupling • heart failure

The transverse tubules (t-tubules) of mammalian cardiac ventricular myocytes are invaginations of the surface membrane that occur at the Z line and have both transverse and longitudinal elements. Many of the proteins involved in excitation-contraction coupling appear to be concentrated at the t-tubules. Therefore, it has been suggested that the t-tubules play a central role in cell activation. In the present review, we will consider the immunohistochemical and functional evidence for protein localization at the t-tubules, potential problems in the interpretation of such data, and the functional consequences of such localization. We will also consider the possible role of the t-tubules in the functional changes that occur during cardiac development, hypertrophy, and failure.

Occurrence and Morphology of the T-Tubules

T-tubules are present in the cardiac tissue of all species of mammals so far investigated (eg, mice, rats, guinea pigs, rabbits, dogs, pigs, and humans) but appear to be absent in avian, reptile, and amphibian cardiac tissue. Within mammalian cardiac tissue, t-tubules occur predominantly in ventricular myocytes, being either absent or far less developed in atrial, pacemaking, and conducting tissue, although a recent report has suggested that ~50% of atrial myocytes possess a sparse irregular tubular system. The following discussion will concentrate on mammalian ventricular myocytes.

The t-tubules are invaginations of the sarcolemma and glycocalyx, which appears to remain associated with the sarcolemma within the t-tubules. Early studies of cardiac muscle showed that they occur at the Z line, at the end of each sarcomere; therefore, they occur at intervals of ~2 μm along the longitudinal axis of the ventricular myocyte. Subsequent studies have shown that the t-tubular system also has longitudinal extensions. Although the t-tubules leave the surface membrane at the Z line, forming an approximately rectangular array, only ~60% of the tubular volume occurs near the Z line; the other 40% occurs between the Z lines. Thus, the t-tubular system is not, as its name might suggest, a simple transverse system of tubules but is a complex system of branching tubules with both transverse and longitudinal elements (Figure 1). Because of its complexity, it has been suggested that the transverse-axial tubular system, sarcolemmal Z rete, or sarcolemmal tubule network might be a more appropriate name. However, t-tubule remains the standard term and will be used in the present review, and surface sarcolemma will be used to describe that part of the cell membrane not within the t-tubules.

Estimates of the percentage of the ventricular myocyte volume occupied by the t-tubules varies from 3.6% in rat myocytes to 0.8% in mouse myocytes, although this variation probably reflects methodological rather than species differences because there appears to be no clear relationship between species and estimates of percentage cell volume occupied by the t-tubules. Similarly, estimates of the percentage of cell membrane located in the t-tubules vary from 64% (calculated in Bers from data in Soeller and Cannell) to 21%, with no apparent species differences.

The t-tubules of cardiac muscle have a mean diameter of ~200 to 300 nm, although within a single rat ventricular myocyte, the diameter of individual tubules can vary from 20 to 450 nm, but with more than half the t-tubules having diameters between 180 and 280 nm. One consequence of this network of narrow tubules is that a rapid change in the composition of the solution around a ventricular myocyte results in a slower change in the composition of the fluid within the t-tubules because of the time required for diffusion...
into the t-tubular network. In guinea pig myocytes, a rapid change of [Ca^{2+}], results in the diffusion of Ca^{2+} into the t-tubules at 3 to 16 μm/s,\(^{16}\) so that the solution change within the t-tubules is delayed by up to 2.3 seconds, and washout of Ca^{2+} from the t-tubules occurs with a time constant of up to 1.7 seconds for the deeper regions of the t-tubular system,\(^{16}\) although these time courses may vary between species,\(^{17}\) possibly reflecting species differences in t-tubule morphology.

Although the diversity of estimates of the extent of the t-tubules within a species makes it impossible to determine whether there may be differences between species, it is tempting to speculate that the extent of the t-tubules varies between species depending on cell size (surface area/volume ratio) and heart rate if the t-tubules are necessary to produce synchronous Ca^{2+} release throughout the cell (see Coupling of Ca^{2+} Entry and Ca^{2+} Release, later) and, hence, synchronous contraction. Although we are unaware of any differences related to cell size, the density of the t-tubular network is greater in the mouse, which has a resting heart rate of 300 to 400 bpm, than in the pig, which has a heart rate of <100 bpm.\(^1\)

### T-Tubule Development Is Labile

Ventricular myocytes isolated from neonatal hearts show little evidence of t-tubule development,\(^4,18\) and cells kept in culture for 6 days show a progressive decrease of t-tubule density.\(^19\) T-tubule structure has also been reported to change in myocytes from failing hearts (see T-Tubule Development and Morphology in Hypertrophy and Failure). However, the mechanisms underlying the expression and maintenance of the t-tubules are not clear. Once formed, the ability of the tubular system to maintain its remarkable degree of structure despite the forces exerted during the normal contraction cycle may be due to the presence of a “scaffold” of focal adhesion molecules, membrane-associated proteins, and basal lamina proteins.\(^20\)

### Proteins Present in the T-Tubule Membrane

The function of the t-tubules depends not only on their structure but also on the proteins within, and adjacent to, the t-tubule membrane. Immunohistochemical techniques have been widely used to investigate the location of proteins within cardiac ventricular myocytes. Such studies have shown marked variations in the distribution of membrane proteins, although a distinction needs to be drawn between the fraction of a particular type of protein located at the t-tubule and whether this is greater than the fraction of the total cell membrane located within the t-tubules, ie, whether protein density is higher (concentrated) at the t-tubule. This is discussed further in Interpretation of Immunohistochemical Data and in Conclusions and Unanswered Questions.

### Ca^{2+}-Handling Proteins

The location of sarcolemmal Ca^{2+}-handling proteins is important because of their role in excitation-contraction coupling and because the Ca^{2+}-release channels (ryanodine receptors [RyRs]) of the sarcoplasmic reticulum (SR) are concentrated close to the t-tubule (see Protein Colocalization Within, and With Proteins Adjacent to, the T-Tubes).

In the rabbit heart, an early study showed that a membrane fraction from the t-tubules had a higher density of L-type Ca^{2+} channels than did membrane from the surface sarcolemma.\(^21\) A more recent immunologic study also found this channel to be concentrated in the t-tubules of adult rabbit ventricular myocytes, with less staining of the surface sarcolemma, although the surface staining that did occur was punctate and associated with junctional SR.\(^22\) In rat heart, the L-type Ca^{2+} channel, and hence current (I_{Ca}), is also concentrated in the t-tubules, with estimates ranging from 3 to 9 times more concentrated in the t-tubule membrane than on the surface sarcolemma.\(^23-26\) Comparative studies suggest that the t-tubular concentration of the L-type Ca^{2+} channel is greater in rat ventricular myocytes than in those from the rabbit.\(^22\)

Although a high density of L-type Ca^{2+} channel at the Z line has been reported in all studies of which we are aware, it has also been reported that in some rat ventricular myocytes, the channel is present in appreciable amounts on the surface membrane,\(^25\) and in sheep and bovine cardiac tissue, it has been reported that this channel is present at a lower concentration on the putative t-tubule membrane than on the surface sarcolemma.\(^27\) However, the majority of recent reports suggest that this channel is concentrated in the t-tubules compared with the surface sarcolemma.

The distribution of the Na^{+}-Ca^{2+} exchanger (NCX) has been more controversial. The first study of its distribution showed it to be predominantly in the t-tubules in guinea pig ventricular myocytes.\(^28\) Subsequent studies (eg, Musa et al\(^25\) and Kieval et al\(^29\)) have shown a more even distribution between the t-tubule and surface membranes, although a recent immunologic study of rat ventricular myocytes also...
showed NCX located predominantly within the t-tubular network. Thus, although there is a consensus that the NCX protein is found in the t-tubule membrane, it is less clear whether this protein is at a higher concentration in the t-tubules or is homogeneously distributed between the t-tubules and surface sarcolemma.

The distribution of other important sarcolemmal Ca\(^{2+}\)-handling proteins, in particular, the sarcolemmal Ca\(^{2+}\)-ATPase and the T-type Ca\(^{2+}\) channel, is currently unknown. However, as pointed out in Takeuchi et al, 34 the resolution of staining in that study was relatively low; subsequently, K\(_{v4.2}\), one of the channels that underlies the transient outward current (I\(_{to}\)) in ventricular myocytes, was shown to be localized predominantly to the t-tubular system. Similarly, the K\(^{+}\) channel TASK-1, 35 which may underlie the steady-state outward current (I\(_{Ks}\)), and K\(_{v2.1}\), 36 which is believed to underlie the inward rectifier K\(^{+}\) current (I\(_{K1}\)), also appear to be predominantly in the t-tubules.

**Anion-Handling Proteins**

In addition to Na\(^{+}\)-H\(^{+}\) exchange, another pH-regulating protein, the Cl\(^{-}\)-HCO\(_{3}\) exchanger, is present, and may be concentrated, at the t-tubule. 37 It is tempting to speculate that it is important that the pH of this region of the cell, which contains so many proteins important for normal cell function, is tightly regulated. 30

**Second-Messenger Pathways**

The β-adrenergic pathway is important in the regulation of a number of key proteins in the heart, in particular, the L-type Ca\(^{2+}\) channel. The mRNA for this channel, the SR Ca\(^{2+}\)-ATPase (via the regulatory protein phospholamban), and the contractile proteins (via troponin I). It has been suggested that there is local regulation by this pathway, particularly of the L-type Ca\(^{2+}\) channel. A structural basis for such regulation is suggested by reports that key elements in this signaling cascade (G\(_i\), adenylate cyclase, and A-kinase anchoring protein) are concentrated at the t-tubule membrane. 39–41 Interestingly, the protein phosphatase calcineurin, which will antagonize the effects of this pathway, appears to be colocatalized with protein kinase A (PKA) near the t-tubules. The β-receptor may also be associated with the L-type Ca\(^{2+}\) channel, because in neurons this receptor has been shown to be part of a macromolecular signaling complex with the L-type Ca\(^{2+}\) channel Ca\(_{V1.2}\), a G protein, and
adenylate cyclase. This could account for the observation that stimulation of this receptor can increase \( I_{Ca} \) without an increase in whole-cell cAMP concentration (see Signal Transduction). Such a localized response has also been shown for the NO synthase 3 pathway.

Interpretation of Immunohistochemical Data
Data from these studies show many key proteins concentrated at the t-tubule (Figure 2). However, there are a number of potential problems with immunohistochemical studies. First, the observed distributions may be artifactual. It has, for example, been suggested that high-intensity staining at the intercalated disks and t-tubules may be a consequence of membrane folding and a high “brightness factor,” respectively, so that quantification of protein distribution from such studies is difficult. Second, the observed distribution may depend on epitope accessibility rather than protein distribution, and spatial resolution is limited; further immunogold labeling may yield more information. Third, it is not always clear that a “striated” pattern of cell staining is colocalized with the t-tubule membrane or that the protein is inserted in the membrane. Therefore, it is difficult to be sure that particular proteins are concentrated at the t-tubules, particularly when the amount of membrane within the t-tubules is also unclear.

It is interesting, however, that so many proteins are identified at the Z line, which, if correct, raises questions of whether these proteins function at the t-tubules or whether they are simply trafficked via this region of the cell. It is not clear that protein location always corresponds to function, partly because of some of the problems mentioned above and partly because protein function will also depend on other factors, such as local environment, second messengers, and accessory proteins. Localization of protein function will be considered further (in Localization of Function). Concentration of many proteins at the t-tubules may reflect their importance in excitation-contraction coupling (see Coupling of Ca\(^{2+}\) Entry and Ca\(^{2+}\) Release), although this will depend on not just the presence of the proteins but their juxtaposition (see Protein Colocalization Within, and With Proteins Adjacent to, the T-Tubules, below).

Protein Colocalization Within, and With Proteins Adjacent to, the T-Tubules
The SR is the major intracellular Ca\(^{2+}\) store in cardiac muscle. Ca\(^{2+}\) entry across the cell membrane triggers the release of further Ca\(^{2+}\) from the SR, via the RyR, and it is predominantly this Ca\(^{2+}\) that causes contraction.

RyRs were first reported as “feet” between the sarcolemma and SR membrane. Immunologic studies have shown a high density of RyRs in the junctional SR (the Ca\(^{2+}\)-release site) adjacent to the t-tubule. Thus, the site of Ca\(^{2+}\) release from the SR appears to be concentrated at the t-tubule, adjacent to the site of transsarcolemmal Ca\(^{2+}\) flux (see Ca\(^{2+}\)-Handling Proteins).

Interestingly, the SR Ca\(^{2+}\) uptake pump (SERCA2), which is responsible for removing Ca\(^{2+}\) from the cell cytoplasm to cause relaxation and which, according to structural and biochemical studies (eg, Jorgensen et al), is thought to be located throughout the SR membrane, has been shown immunohistochemically to be concentrated at the Z line, adjacent to the t-tubule. The regulatory protein phospholamban, which modulates the activity of SERCA2, shows a similar distribution (F. Brette, unpublished data, 2002).

Immunologic investigation of colocalization of proteins involved in excitation-contraction coupling has shown that Ca\(^{2+}\) channels are highly colocalized with RyRs in the t-tubules, forming the dyad. However, NCX shows little colocalization with either the RyR or Na\(^{+}\) channel. Therefore, it has been suggested that the t-tubule can be subdivided into 3 domains: (1) the dyad, (2) one containing the Na\(^{+}\) channel, and (3) one containing NCX. This suggests that the most effective functional coupling will be between the L-type Ca\(^{2+}\) channel and the RyR, although the mechanism of colocalization is still unknown. The lack of colocalization of NCX with RyR may be one reason why Ca\(^{2+}\) influx via NCX is a less effective trigger for SR Ca\(^{2+}\) release. The lack of colocalization of NCX and Na\(^{+}\) channels is also interesting, because it has been suggested that Na\(^{+}\) influx via Na\(^{+}\) channels may enhance Ca\(^{2+}\) entry on the exchanger, a mechanism that would presumably require colocalization; however, the proximity of NCX to junctional SR may allow NCX activity to be modulated by Ca\(^{2+}\) released from the SR (see Trafford et al and Ca\(^{2+}\) Efflux).

Localization of Function
The studies described above suggest that many membrane proteins are concentrated at the t-tubules. However, protein function will not necessarily reflect protein distribution (above). A different approach has been to investigate the localization of function by using a number of techniques, which follow.

Cells Lacking T-Tubules
Some cardiac cells do not have a t-tubular system or have only a very sparse system; these include ventricular myocytes from embryonic and newborn animals, atrial cells, and Purkinje cells. In addition, adult ventricular myocytes kept in culture lose their t-tubular system. A number of studies have investigated the function of these cell types, which may help our understanding of t-tubule function. In myocytes lacking t-tubules (from the ventricles of newborn rabbits, atrial cells, rabbit Purkinje cells, and cultured ventricular myocytes), electrical stimulation causes a rise of [Ca\(^{2+}\)], that occurs initially at the cell periphery and propagates into the cell interior by propagated Ca\(^{2+}\)-induced Ca\(^{2+}\) release in atrial and cultured cells and by diffusion in neonatal and Purkinje cells, and localized Ca\(^{2+}\)-release events (Ca\(^{2+}\) sparks) occur predominantly close to the surface sarcolemma in these cells. This is in contrast to adult ventricular myocytes, in which Ca\(^{2+}\) release occurs synchronously across the width of the cell on electrical stimulation, and Ca\(^{2+}\) sparks arise predominantly at the t-tubule. These data are consistent with the notion that t-tubules play an important role in causing synchronous Ca\(^{2+}\) release in the adult ventricular myocyte, because of the concentration of L-type Ca\(^{2+}\) channels and RyR at the t-tubule (see Ca\(^{2+}\)-Handling Proteins, above), although a problem
with this type of study for the investigation of t-tubule function is that protein expression, as well as t-tubule structure, may be different in these cells.

An alternative approach has been to correlate the loss of t-tubules with loss of membrane currents. During 4 days in culture, the membrane capacitance of rabbit ventricular myocytes, taken to represent membrane area, decreases by 51% with a time course that correlates with the loss of 83% of $I_{Ca}$ and 88% of the ATP-sensitive $K^+$ current ($I_{KATP}$), suggesting that these currents are concentrated in the t-tubules. However, another study has shown more complicated changes in rabbit ventricular myocytes during culture: although t-tubule density and cell capacitance decrease continuously during 6 days in culture, Ca$^{2+}$ channel density decreases by ~50% after 1 day and then partially recovers, $I_{Ca}$ also declines by ~50% within 1 day but shows no recovery, whereas $I_{K}$ changes little in 1 day but decreases by 65% after 6 days, emphasizing the difficulty of distinguishing between changes due to loss of the t-tubules and changes in protein expression.

**Diffusion Studies**

A different approach has been to investigate the rate of change of membrane currents in ventricular myocytes after a rapid change in perfusate composition. Because of the long diffusion time into the t-tubules (see Occurrence and Morphology of the T-Tubules), currents on the cell surface would be expected to change rapidly, whereas those located in the t-tubules would be expected to change over a longer time course. By use of this technique, it has been shown that the time course of $K^+$ current inhibition by Ba$^{2+}$ is consistent with a model that includes a diffusion time constant of 300 ms, consistent with much of $I_{K1}$ and $I_{KATP}$ being localized in the t-tubules. A similar approach has been used in guinea pig myocytes to investigate the time course of changes of the Na$^+$ current ($I_{Na}$) and $I_{Ca}$ in response to rapid changes of extracellular Na$^+$ and Ca$^{2+}$, respectively. In atrial cells, which lack t-tubules, changing the bathing Na$^+$ or Ca$^{2+}$ produced rapid changes of $I_{Na}$ and $I_{Ca}$ (time constant of ~25 ms). However, in ventricular myocytes, only 36% of the current changed rapidly; the remaining 64% of current changed with a current constant of ~200 ms, suggesting that this percentage of $I_{Na}$ and $I_{Ca}$ is within the t-tubules. Interestingly, this percentage is the same as the upper estimate of the percentage of cell membrane within the t-tubules (see Occurrence and Morphology of the T-Tubules), which would imply that these currents are found predominantly, but not concentrated, within the t-tubules.

**Scanning Pipette**

An elegant technique has been described recently that uses a pipette to scan the cell surface and simultaneously monitor membrane currents. To date, this technique has been used to investigate the location of $I_{KATP}$, which appears to be concentrated in the vicinity of the Z line, consistent with the proposal (see Diffusion Studies) that this current is found predominantly in the t-tubule. However, it is not clear whether the current monitored at the Z line by this technique simply reflects the amount of membrane under the electrode (ie, in the t-tubule) or whether the current is concentrated on the surface sarcolemma at the Z line.

**Detubulation**

Another approach developed recently has been to adapt the “osmotic shock” technique used previously to detubulate skeletal muscle to disrupt the t-tubules of rat ventricular myocytes. Detubulation decreases cell capacitance by ~30%. It is not clear that the standard method used to measure membrane capacitance (eg, 10-mV 10-ms hyperpolarizing pulses from -80 mV) monitors the capacitance of all the t-tubule membrane (see Electrical Properties of T-Tubules). However, assuming the capacitance of the t-tubule membrane to be the same as that of the surface sarcolemma, this gives a lower limit to the percentage of the cell membrane found within the t-tubules.

Most (~87%) of $I_{Na}$ and almost all NCX activity are lost after detubulation, suggesting that the function of both of these Ca$^{2+}$ flux pathways is concentrated in the t-tubules of rat ventricular myocytes, although if cell capacitance is underestimated more than tonic currents, it is possible that these currents are concentrated less than these measurements might suggest (see Electrical Properties of T-Tubules). In contrast, $I_{K1}$ shows uniform distribution between the t-tubule and surface membranes, as do $I_{Kc}$, $I_{Na}$, and $I_{Ca}$, although $I_{K}$ appears to be concentrated in the t-tubules. This distribution of $I_{K}$ agrees with immunohistochemical studies of TASK-1 (see K$^{+}$-Handling Proteins). However, the proteins thought to underlie $I_{Na}$ and $I_{K}$ appear to be concentrated in the t-tubules (see K$^{+}$-Handling Proteins); this discrepancy may be due to the difficulties of these techniques (see Interpretation of Immunohistochemical Data, above, and Electrical Properties of T-Tubules, below) or to local regulation or differential distribution of accessory proteins such as K$^{+}$-4.2, which, with K$^{+}$-4.2a,b, forms functional $I_{Na}$ channels.

Detubulation shows a greater percentage of Ca$^{2+}$ flux via $I_{Ca}$ and NCX in the t-tubules than might be expected from immunohistochemical studies. One way of reconciling these data is to suggest that the protein in the t-tubule is more active than that in the surface membrane (because of stimulation of $I_{Ca}$ by second-messenger pathways, for example), so that relatively more function is lost than protein after detubulation. In support of this idea, $\beta$-adrenergic stimulation of $I_{Ca}$ causes a greater increase in current in normal cells than in detubulated cells, suggesting that $I_{Ca}$ in the t-tubules might be better coupled to this second-messenger pathway than that in the surface membrane. In the presence of tonic activity of this pathway, this could explain the relatively large loss of current after detubulation.

**Functional Role**

**Electrical Properties of T-Tubules**

Localized depolarization of skeletal muscle at the t-tubule causes contraction of the adjacent half sarcomeres. By analogy, it has been assumed that the t-tubules in cardiac myocytes allow propagation of excitation into the cell to cause synchronous activation, although analogous experiments in cardiac muscle have not shown localized activation,
possibly because the longitudinal extensions of the t-tubules allow the spread of excitation to adjacent regions of the cell.66

Tidball et al67 observed that the response of the action potential of rabbit ventricular trabeculae to the SR inhibitor ryanodine was correlated with the degree of t-tubule development and postulated that the t-tubules had a density of Ca2+ and K+ channels that was different from that of the surface membrane. It now appears likely that there are at least 3 factors that could contribute to differences between the t-tubule and surface membrane action potentials: (1) many of the membrane proteins that underlie the action potential are unevenly distributed between the surface and t-tubule membranes (above), so that the electrophysiology of the t-tubules would be expected to differ from that of the surface membrane; (2) modeling of skeletal muscle t-tubules has shown that 2 to 3 ms is required before the potential throughout a simple t-tubule model matches that at the cell surface;68 and the complex structure of the cardiac t-tubular system may result in longer delays; and (3) the t-tubules form a restricted extracellular space that will allow ion accumulation and depletion, which can modify electrical activity (see Clark et al69). In isolated cells, this may differentially alter the t-tubule action potential; in the intact muscle, diffusion is also restricted in the intercellular clefts, and the extent to which diffusion from the t-tubules and the intercellular clefts will differ is unknown.

Consideration of the electrical properties of the t-tubules is important (1) because it seems likely that the t-tubules are the most important site for excitation-contraction coupling and (2) because standard voltage-clamp techniques may not effectively clamp potential, and hence monitor membrane currents, within the t-tubular system.69 Thus, ionic currents at the surface membrane may be more effectively monitored than those in the t-tubules, so that loss of currents with loss of t-tubules (see Cells Lacking T-Tubules and Detubulation) may be underestimated. Similarly, since the voltage drop down the t-tubules is unknown, it is possible that measurements of cell capacitance (see Cells Lacking T-Tubules and Detubulation) underestimate the percentage of cell membrane within the t-tubules. If capacitance and membrane currents are underestimated to a similar degree, calculation of the concentration of these currents in the t-tubules will be the same. However, if cell capacitance is underestimated more than ionic currents, these currents may be less concentrated in the t-tubules than suggested by such measurements.

**Coupling of Ca2+ Entry and Ca2+ Release**

The data presented above suggest that \( I_{Ca} \) and NCX activity occur predominantly in the t-tubules, close to the RyR, implying that the t-tubules play a central role in Ca2+ cycling and excitation-contraction coupling in cardiac ventricular myocytes. Although \( I_{Ca} \) is more effective than NCX in triggering SR Ca2+ release,50,51 probably because the L-type Ca2+ channel, unlike NCX, is colocalized with RyR26 and because of the higher Ca2+ flux through the channel,70 the presence of NCX in the t-tubules means that Ca2+ flux via NCX may participate in the process.71

The local control theory of Ca2+ release (see Wier and Balke47 for review) is that local Ca2+ entry across the cell membrane, predominantly via \( I_{Ca} \) triggers local Ca2+ release from an adjacent cluster of RyRs. The whole-cell Ca2+ transient is the temporal and spatial sum of these individual localized release events.72 Ca2+ sparks occur predominantly near the t-tubules,59,73 and localized Ca2+ release (Ca2+ spikes) occurs at discrete sites at the Z line.74 These spikes (Figure 3) are proportional to \( I_{Ca} \) and to derived SR Ca2+ flux, providing strong support for the idea that local Ca2+ entry across the t-tubule membrane triggers local Ca2+ release from adjacent RyRs.

The importance of the Ca2+ release occurring at the t-tubules has been demonstrated in cells lacking t-tubules in which the electrically stimulated rise of \([Ca^{2+}]_i\) initially occurs close to the cell membrane and then either diffuses (Purkinje64 and neonatal6 cells) or propagates via Ca2+-induced Ca2+ release (atrial,75 cultured,56 and detubulated75,58 cells) into the cell (Figure 4). Interestingly, in detubulated cells, the speed of propagation is increased by β-adrenergic stimulation76 so that synchronization of Ca2+ release is increased77 even in the absence of t-tubules.76 In addition, compared with pig myocytes, mouse myocytes show a rapid and synchronous rise of \([Ca^{2+}]_i\).78 This difference appears to be due to the higher t-tubule density in mouse myocytes. Therefore, it appears that Ca2+ release occurs predominantly at the t-tubules, which therefore underlie the temporally and spatially synchronous Ca2+ release observed in ventricular myocytes (see Yang et al58).
Although NCX is well recognized as an important Ca\textsuperscript{2+} efflux pathway (see Ca\textsuperscript{2+} Efflux), the suggestion that Ca\textsuperscript{2+} entry on NCX at the start of the action potential might trigger SR Ca\textsuperscript{2+} release\textsuperscript{28} has been more controversial; most studies suggest that it is not as effective as \( I_{\text{ca}} \) (above) and may not be the normal physiological trigger but rather modulates the trigger effect of \( I_{\text{ca}} \) and/or acts as a trigger only under certain conditions. It has been proposed that Ca\textsuperscript{2+} entry on the exchanger, and hence its ability to act as a trigger, is enhanced by colocalization with \( I_{\text{ca}} \) which will increase the [Na\textsuperscript{+}] in a submembrane microdomain (fuzzy space\textsuperscript{79}) that is sensed by NCX. However, Scriven et al\textsuperscript{26} have shown that the L-type Ca\textsuperscript{2+} channel, NCX, and \( I_{\text{ca}} \) are in different microdomains, although a demonstration that brain-specific isoforms of the Na\textsuperscript{+} channel are concentrated in the t-tubules\textsuperscript{32} may require the colocalization of these channels with other proteins in the t-tubule to be investigated.

As well as “feed-forward” from Ca\textsuperscript{2+} entry via L-type Ca\textsuperscript{2+} channels and NCX to the RyR, the proximity of these proteins suggests that Ca\textsuperscript{2+} released from the SR may “feed back” onto the sarcosomal proteins concentrated at the t-tubule (see Ca\textsuperscript{2+} Efflux, below).

**Ca\textsuperscript{2+} Efflux**

There are 2 sarcosomal Ca\textsuperscript{2+} efflux pathways in ventricular myocytes: NCX and sarcosomal Ca\textsuperscript{2+}-ATPase. The exchanger appears to be localized within the t-tubules and is therefore close to the site of SR Ca\textsuperscript{2+} release (above); the distribution of Ca\textsuperscript{2+}-ATPase is known, although it is present on the surface membrane.\textsuperscript{58} There are at least 2 lines of evidence to support the idea that Ca\textsuperscript{2+} released from the SR has “privileged” access to NCX. First, when Ca\textsuperscript{2+} is released from the SR using caffeine, there is hysteresis between bulk [Ca\textsuperscript{2+}], and NCX current, with a given current being produced by a lower bulk [Ca\textsuperscript{2+}], as [Ca\textsuperscript{2+}] is increasing, suggesting that Ca\textsuperscript{2+} is released close to NCX, resulting in a higher local [Ca\textsuperscript{2+}], that stimulates NCX.\textsuperscript{53} Second, Ca\textsuperscript{2+} efflux appears to occur during systole, compatible with Ca\textsuperscript{2+} release occurring close to NCX.\textsuperscript{90} Interestingly, recent immunohistochemical work has shown that SR Ca\textsuperscript{2+}-ATPase also appears to be localized close to the t-tubule.\textsuperscript{25}

The observation that major Ca\textsuperscript{2+} sequestration pathways are located at the t-tubule raises the question of why this is so. The presence of NCX in the t-tubules may allow rapid Ca\textsuperscript{2+} efflux throughout the cell, thus helping to produce synchronous relaxation. However, it also appears that this arrangement will produce futile Ca\textsuperscript{2+} cycling. The reason for this is unknown, although it has been suggested that by regulating [Ca\textsuperscript{2+}] close to the RyR, NCX may alter the threshold for, and thus help regulate, SR Ca\textsuperscript{2+} release.\textsuperscript{71}

**Signal Transduction**

Colocalization of PKA and calcineurin occurs at the t-tubules (see Second-Messenger Pathways), and a local increase in cAMP along the Z lines has been shown by using fluorescence resonance energy transfer (Zaccolo and Pozzan\textsuperscript{88}). These authors suggested that a localized increase of cAMP occurs near the t-tubules, although their preparation, embryonic cardiac cells, lacks t-tubules. However, this suggests that the Z line, rather than the t-tubules, might provide the scaffold for the cell structure in this region, and further experiments in adult ventricular myocytes would be of interest.

There is also functional evidence for a local elevation of cAMP and subsequent protein phosphorylation. Xiao\textsuperscript{82} has provided evidence that \( \beta \)-adrenergic stimulation can produce an increase in \( I_{\text{ca}} \) without causing phosphorylation of other proteins, such as phospholamban, compatible with local signaling and close association of the \( \beta \) signaling pathway with the L-type Ca\textsuperscript{2+} channel (see Second-Messenger Pathways). The observation that the L-type Ca\textsuperscript{2+} channel is less sensitive to \( \beta \)-adrenergic stimulation in detubulated cells, although phospholamban phos-
phorylation is unaffected,\textsuperscript{83} suggests that this local signaling occurs predominantly in the t-tubules.

**Changes During Development and Disease**

Data from normal cells suggest that the major difference between the t-tubule and surface membranes is the concentration of Ca\textsuperscript{2+}-handling proteins at the t-tubules. Therefore, in this section, we will concentrate on these proteins.

**T-Tubule Development and Morphology in Hypertrophy and Failure**

Embryonic and neonatal cardiac myocytes lack t-tubules,\textsuperscript{5,84} which develop during the first few weeks of life, with the precise time of development differing between species. In addition, during cell culture (above) and heart failure (below), t-tubule morphology changes, indicating that t-tubule structure is labile even in the mature cell.

How the t-tubules develop and are maintained is less clear. Lee et al\textsuperscript{85} have shown that expression of amphiphysin-2, a protein that can link the plasma membrane and submembranous cytosolic scaffolds in CHO cells, generates narrow tubules that are continuous with the plasma membrane. The t-tubule membrane appears to have a distinct protein and lipid composition and is enriched in cholesterol, which can be used as a tool to separate t-tubule and surface membranes.\textsuperscript{86} The development of the t-tubules appears to depend on protein and lipid and shows properties that are similar to the development of caveolae, which requires cholesterol and caveolin-3.\textsuperscript{87} T-tubules are composed of interconnected caveola-like elements, and repeated caveolae formation in the absence of fission leads to the generation of t-tubules.\textsuperscript{88,89} Treatment with amphotericaerin B, an antibiotic that binds cholesterol, causes the disruption of t-tubules in C\textsubscript{6}C\textsubscript{12} myotubes.\textsuperscript{86} This does not exclude the existence of "true" caveolae (50- to 80-nm-diameter invaginations, see Razani et al\textsuperscript{87} for review) along the surface sarclemma and t-tubule membrane.\textsuperscript{11}

Despite the evidence that the t-tubules are important in excitation-contraction coupling, there have been relatively few studies of this network during pathological conditions. An early morphological study showed that hypertrophy of rat left ventricle, produced by aortic constriction, results in an increase in the t-tubule area, which helps maintain the surface area/volume ratio of the hypertrophied cells.\textsuperscript{90} However, in rat doxorubicin-induced cardiomyopathy, cell capacitance decreased significantly, possibly as a result of t-tubule damage.\textsuperscript{91} To date, only one study has examined t-tubule structure in pathological living cells. He et al\textsuperscript{92} showed that in canine tachycardia-dilated cardiomyopathy, t-tubules are lost at each extremity of the cell but remain intact in the center of the cell (interestingly, a similar pattern of t-tubule loss has been reported during culture\textsuperscript{19}); however, cell capacitance increased by 13%, possibly because of a hypertrophy-induced increase in surface sarclemma compensating for the loss of t-tubules.

Data from the human heart is equally equivocal. In hypertrophic human heart, the t-tubules appear to be aberrantly shaped\textsuperscript{92} or dilated.\textsuperscript{20} Dilation has been also observed in failing human heart,\textsuperscript{93} although the changes were not quantified in either study. In contrast, a preliminary study of human ventricular myocytes suggests that t-tubule density is not altered in failing hearts,\textsuperscript{94} whereas another preliminary report showed decreased t-tubule density in myocytes from failing human ventricle.\textsuperscript{6}

The diversity of results from animal and human studies may reflect the diversity of models and conditions studied. Further investigation of the development, morphology, and composition of the t-tubules from the failing heart may elucidate both the regulation of t-tubule structure\textsuperscript{5,92} and its role in the changes in function observed in pathological conditions (see Gomez and colleagues\textsuperscript{95,96} and Relevance of Changes in T-Tubule Structure and Protein Expression to Function).

**Changes in Protein Expression in Development, Hypertrophy, and Failure**

Ventricular myocytes from newborn animals show little t-tubule development (see T-Tubule Development and Morphology in Hypertrophy and Failure) and relatively little SR\textsuperscript{97} but enhanced I\textsubscript{Ca} and NCX activity\textsuperscript{99} compared with adult cells. It seems likely that these cells rely mainly on extracellular Ca\textsuperscript{2+} for activation.\textsuperscript{100} The transition to the more mature (SR-dominated) form of excitation-contraction coupling during the first few weeks of life is accompanied by SR development, decreased I\textsubscript{Ca} and t-tubule formation (Haddock et al\textsuperscript{4} and Chen et al\textsuperscript{18}), and NCX at least has been reported to appear in the t-tubules as soon as they are formed.\textsuperscript{18}

Ventricular myocytes from failing hearts show reduced contraction, blunted \(\beta\)-adrenergic responsiveness, and myocyte hypertrophy. For review, see Tomaselli and Marban\textsuperscript{101}; we will concentrate on changes in the proteins that occur at the t-tubules.

A decrease in the amplitude and the rate of decline of the systolic Ca\textsuperscript{2+} transient is a consistent finding in failing heart muscle. This appears to be due to depressed SERCA2 activity, which will slow the rate of decline of the Ca\textsuperscript{2+} transient and decrease SR Ca\textsuperscript{2+} content. In addition, RyRs may be hyperphosphorylated, increasing Ca\textsuperscript{2+} leak\textsuperscript{102} and reducing SR Ca\textsuperscript{2+} content.

Most reports show no change in the density of I\textsubscript{Ca} during hypertrophy and failure.\textsuperscript{103} This may be due to the presence of fewer channels, with upregulation of the remaining channels,\textsuperscript{5,104} consistent with work showing an increase in the activity of single channels from failing human ventricular myocytes.\textsuperscript{105} This upregulation may be due to channel phosphorylation,\textsuperscript{104} which may also explain, at least in part, the blunted response of failing hearts to \(\beta\)-adrenergic stimulation.

NCX expression and current density appear to be increased in hypertrophy and failure,\textsuperscript{106} although it has recently been shown that Ca\textsuperscript{2+} transport via NCX is virtually unchanged in rat ventricular myocytes after myocardial infarction if NCX function is normalized to cell volume.\textsuperscript{107}

Thus, there have been many studies of the changes that occur in the hypertrophied and failing heart and, in particular, of proteins that are normally found at the t-tubules. There is also evidence that the t-tubule structure changes in these conditions. However, there is a paucity of data regarding whether these changes are associated with changes in the distribution of proteins. It is tempting to speculate that remodeling of the t-tubules and changes in Ca\textsuperscript{2+}-handling protein expression and distribution might alter excitation-contraction coupling in failing ventricular myocytes.
Relevance of Changes in T-Tubule Structure and Protein Expression to Function

Myocytes from newborn animals have a relatively large surface area/volume ratio, and myofilaments are located in the subsarcolemmal region. It appears likely that sufficient Ca\(^{2+}\) can enter via the surface membrane to activate the myofilaments. However, these cells also show marked gradients of Ca\(^{2+}\) during excitation-contraction coupling: Ca\(^{2+}\) rises initially at the cell periphery and then diffuses into the center of the cell. The development of the t-tubules is associated with a transition to the mature pattern of excitation-contraction coupling, which shows a synchronous increase of [Ca\(^{2+}\)] across the cell.

The ability of \(I_{Ca}\) to trigger Ca\(^{2+}\) release from the SR is reduced in myocytes from failing rat hearts. It has been suggested that this may be due to changes in the colocalization of the L-type Ca\(^{2+}\) channel and RyR, increased separation of the t-tubule from the SR, or t-tubule remodeling. A confounding factor is that 4 different isoforms of the \(\alpha_{1C}\) subunit of the L-type Ca\(^{2+}\) channel are expressed in the normal human heart, and isoform switching occurs in failing human myocytes. In addition, several lines of evidence indicate hyperphosphorylation of PKA target proteins in human heart failure, and because key target proteins appear to be localized at the t-tubules (above), changes in protein phosphorylation at the t-tubules may also underlie changes in excitation-contraction coupling.

However, many of these proposed mechanisms rely on a change in the efficiency with which \(I_{Ca}\) triggers SR Ca\(^{2+}\) release. It has been cogently argued that such a mechanism can produce only short-lived changes in the size of the Ca\(^{2+}\) transient and that it is more likely that a decrease in SR Ca\(^{2+}\) content underlies the decrease in the Ca\(^{2+}\) transient. However, it remains possible that a reduction in t-tubule density in heart failure could desynchronize Ca\(^{2+}\) release on electrical stimulation, reducing peak [Ca\(^{2+}\)], and slowing its time course.

The location of NCX is also of interest because the activity of this protein appears to be upregulated in heart failure, which can improve contractile function but may also lead to arrhythmias; however, it is unclear whether it retains its t-tubular location during failure.

Conclusions and Unanswered Questions

It is clear that the t-tubules are not simple invaginations of the sarcolemma. Ca\(^{2+}\)-handling proteins in particular appear to be located predominantly within the t-tubules, which therefore play a central role in excitation-contraction coupling, functioning as the major site for Ca\(^{2+}\) entry and release, allowing synchronous Ca\(^{2+}\) release throughout the cell and Ca\(^{2+}\) removal from the cytoplasm. The t-tubules may also be an important site for modulation of contraction via NCX. What is less clear is whether the density of these proteins is greater within the t-tubules than at the surface membrane, which has important implications for protein trafficking, or whether the fraction of these proteins within the t-tubules simply reflects the fraction of membrane within the t-tubules. Accurate estimates of protein and membrane fraction are required, although it is notable that despite the problems associated with immunohistochemical and electrophysiological investigation of the t-tubules, both techniques show apparent concentration of some proteins within the t-tubules, and many ultrastructural studies agree with capacitance measurements of the amount of cell membrane located in the t-tubules.

There are, however, many other questions that have not been fully answered: how are the t-tubules maintained, and how and why do they change during development, hypertrophy, and heart failure? How are the proteins concentrated at the t-tubules during failure? How are the proteins targeted to this part of the cell membrane? How does protein distribution change during development, hypertrophy, and failure, and what role does this play in the altered function observed in these conditions? What are the electrical properties of the t-tubules, and why are Ca\(^{2+}\)-efflux pathways located close to Ca\(^{2+}\)-release sites? These and many other questions will provide a future challenge in elucidating and understanding the role and the importance of the t-tubules in health and disease.

Acknowledgments

The authors acknowledge financial support from the Wellcome Trust and British Heart Foundation and thank Dr Richard Sainson for helpful discussion and Tim Lee for the preparation of Figure 2.

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Circ Res. 2003;92:1182-1192
doi: 10.1161/01.RES.0000074908.17214.FD
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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