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COMMENTARY

Huntington's disease skeletal muscle has altered T-tubules

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Transverse tubules (T-tubules) are an important determinant of a cell function, especially as the main site of excitation–contraction (EC) coupling. T-tubules ensure spatially and temporally synchronous Ca^{2+} release throughout the striated muscle cell (Hong and Shaw, 2017; Hong et al., 2014). In an earlier issue of the *Journal of General Physiology*, Romer and colleagues used a mouse model of Huntington's disease (HD) to explore a primary skeletal myocyte origin component of HD. The hypothesis tested in the study is that T-tubules are altered in HD skeletal muscle, leading to EC coupling changes and muscle dysfunction (Romer et al., 2021).

HD is a progressive and invariably fatal neuromuscular degenerative disorder (McColgan and Tabrizi, 2018). While much of HD sequelae is associated with neurodegeneration, primary skeletal myopathy has also been suggested (Zielonka et al., 2014). Pathological changes in HD skeletal muscle include metabolic and mitochondrial defects (Lodi et al., 2000; Turner et al., 2007), atrophy (Ehrnhoefer et al., 2014; She et al., 2011), impaired contraction (Hering et al., 2016), and altered expression of genes needed for muscle differentiation (Luthi-Carter et al., 2002). In fact, in an HD human case study, reduced muscle performance has been reported to occur before the presentation of neurological symptoms (Kosinski et al., 2007).

In the present study, Romer et al. (2021) used a R6/2 mouse model of HD to explore primary pathogenesis in skeletal muscle. They previously reported that R6/2 HD skeletal muscle fibers have a decrease in specific membrane capacitance (Miranda et al., 2017). This decrease in capacitance points to a disruption and membrane loss of the T-tubule system which would contribute to changes in EC coupling, Ca^{2+} homeostasis, and muscle function.

In a surprise finding, the authors of this follow up study (Romer et al., 2021) did not detect a difference in T-tubule density, spacing, or regularity between R6/2 and control skeletal fibers. However, R6/2 skeletal muscle fibers had smaller cross-sectional areas and openings (Romer et al., 2021). Thus, T-tubules are not disrupted as much as they are smaller. A reduction in T-tubule size can at least partially account for reduced total cellular membrane capacitance. Furthermore, R6/2 muscle fibers had increased spacing between SR terminal cisternae and T-tubule membranes (Romer et al., 2021), which will contribute to impaired EC coupling. T-tubule and SR docking may precede the incorporation of the RYRs into SR membrane, and especially that of the newly formed T-tubule–SR junctions (Takekura et al., 2001). Impaired T-tubule–SR spacing will affect organization of RYRs among other essential calcium handling proteins.

To explore whether the smaller T-tubules could account for the reduced membrane capacitance in R6/2 fibers, the authors used mathematical modeling to quantify theoretical current flow along T-tubules microdomains. Previous studies of impaired membrane excitability of R6/2 HD skeletal muscle revealed that specific membrane capacitance, when normalized to fiber surface area, progressively decreases in parallel with disease progression (Miranda et al., 2017; Waters et al., 2013). The mathematical model supports that resistance to current along the lumen of T-tubules generates a local voltage drop that effectively lowers specific capacitance. Despite the success of the model in accurately describing T-tubule voltage changes as measured by other methods such as optical mapping, the model only partially explains the reduced capacitance of R6/2 muscle.

After the initial surprise that T-tubules are not disrupted but narrowed, the rest of the findings remain within current understanding of T-tubule maintenance and organization of EC-coupling machinery. Reductions in both T-tubule diameter and increased spacing between SR terminal cisternae and T-tubule membranes will negatively affect EC coupling, overall Ca^{2+} homeostasis, and muscle function. After identifying smaller T-tubules, the authors explored putative candidates for T-tubule membrane organization and T-tubule membrane docking with SR membrane. In particular, the authors took a candidate gene approach, exploring expression of known proteins Bridging Integrator 1 (Bin1) and the Junctophilins (JPH1 and JPH2). Both JHP1 and JHP2 are coexpressed in the triads of skeletal muscle, but only the latter in cardiac muscle.

In the skeletal muscle of the R6/2 mouse model (Romer et al., 2021), Bin1 was found to be reduced as was JPH2, yet a splice

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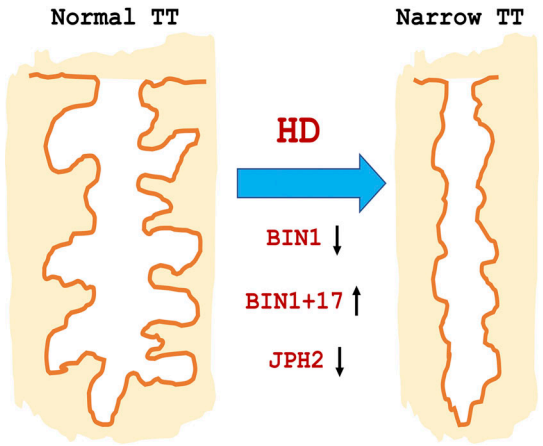

Figure 1. **Schematic representation of T-tubule (TT) before (left) and after (right) possible changes with HD in skeletal muscle.** To explain smaller T-tubule openings yet decreased membrane capacitance in HD skeletal muscle, there could be an abundance of proximal T-tubule membrane yet loss T-tubule folds. Such changes in membrane organization could be a result of altered regulation of T-tubule–associated proteins BIN1 and Junctophilin.

variant of BIN1 that includes exon 17 was increased as summarized in Fig. 1 (Romer et al., 2021). JPHs contribute to SR and T-tubule structural association and reduced expression of these proteins impacts formation and morphology of SR–T-tubule junctions in both skeletal and cardiac muscle (Komazaki et al., 2002). Bin1 has been implicated in skeletal T tubulogenesis (Lee et al., 2002), and its mutation is causative of a skeletal muscular dystrophy (Al-Qusairi and Laporte, 2011). The isoforms associated with skeletal T tubulogenesis include exons 11 and 17, and the finding of increased exon 17 may explain the different structure of the T-tubules while overall Bin1 levels are diminished. In cardiac muscle, loss of Bin1 does not eliminate T-tubules but results in wider and smoother T-tubules (Hong et al., 2014). This effect was mapped, in cardiac muscle, to a Bin1 isoform containing exon 13 and 17 (Hong et al., 2014). An increase in skeletal Bin1 which includes exon 17 would therefore be consistent with smaller T-tubule openings in the setting of an increase in exon specific skeletal Bin1 isoforms. Our knowledge of the role of distinct Bin1 isoforms in skeletal T-tubule generation and maintenance remains in its infancy, and the phenomenon of smaller T-tubules in the setting of overall lower Bin1 yet higher isoforms with exon 17 in R6/2 myocytes is an attractive model to better understand the roles of different Bin1 isoforms in regulating T-tubule membranes.

In general, it should be emphasized, however, that despite earlier reports of Bin1 in skeletal T tubulogenesis in cell lines and isolated skeletal muscle cells (Lee et al., 2002), more recent studies have not been confirmative (Manfra et al., 2017). We are not aware of an in vivo model, whether a disease or genetic intervention such as Bin1 knockout, in which an absence of skeletal T-tubules has been reported. As mentioned above, in cardiac muscle the question relating Bin1 to cardiac T tubulogenesis was directly explored by asking whether Bin1 knockout would result in a lack of cardiac T-tubule generation, and the finding was that Bin1 is not responsible for cardiac T tubulogenesis (Hong et al., 2014). However, that cardiac isoform of Bin1 (cBIN1, which includes exons 13 and 17) is responsible for generating folds of T-tubule membrane (Hong et al., 2014; Zhou and Hong, 2017), which form microdomains that facilitate the trafficking and organization of Cav1.2 (L-type voltage gated channels; Hong et al., 2012), and other Ca^{2+} handling proteins (Hong et al., 2010; Fu et al., 2016; Liu et al., 2020). A loss of cBIN1 results not in smaller T-tubules, but in larger T-tubules without the cBIN1 generated microdomains (Liu et al., 2020). It is therefore surprising that in the current study (Romer et al., 2021), less skeletal Bin1 results were not found in larger or disrupted T-tubules, but in narrower T-tubules. The authors could have performed the high-resolution electron microscopy to explore the presence or absence of microdomains. A loss of microdomain membrane could also explain a loss of overall membrane capacitance. It also is possible, as Romer et al. (2021) suggest, that cardiac myocytes have microdomains, whereas skeletal muscle cells do not. In skeletal muscle, a microdomain is usually a restricted subcellular space between the membranes of two different systems or of two organelles (i.e., the confined space in proximity of Ca^{2+} release sites, or the confined domain of the mitochondrial and SR membranes). This very small volume domain may determine, usually in specific and controlled circumstances, a higher-concentration level of proteins, molecules, or ions, which in turn facilities a preferential functional pathway such as mitochondrial Ca^{2+} uptake (Boncompagni et al., 2009). In cardiac muscle, microdomains are formed by T-tubule folds alone (Hong et al., 2014).

It has also been reported that exercise induces a dynamic assembly of new intracellular junctions called calcium entry units (CEUs) in skeletal muscle. CEUs involve elongation of T-tubule and its association with SR stacks. The formation of these junctions determines enhanced Ca^{2+} influx via store-operated Ca^{2+} entry (SOCE), which improves SR Ca^{2+} release for maintaining contractility during fatigue (Boncompagni et al., 2017; Michelucci et al., 2019). As the assembly of these new SR–T-tubule junctions (CEUs) is primarily dictated by the remodeling of T-tubule (i.e., extension and retraction), it would be plausible that reduction of muscle performance in R6/2 mouse model of HD might be the result of a lowering of both T-tubule plasticity and ability to recover Ca^{2+} ions for the extracellular space via SOCE, even if the role of Bin1 (or of JHPs) in CEUs assembly has been not yet investigated but only speculated (Protasi et al., 2020).

Because Romer et al. (2021) identified a skeletal Bin1 isoform which contains exon 17 was increased, not decreased, it is possible that skeletal Bin1 containing exon 17 increased T-tubule membranes and narrows T-tubule openings. Furthermore, given the tools to explore the roles of different Bin1 isoforms and T-tubule morphogenesis, future studies are encouraged to test for the presence of skeletal T-tubule microdomains directly.

Ultimately, such as is the case with microdomains, a comparison is needed between the makeup of cardiac T-tubules and that of skeletal T-tubules. Classically, cardiac T-tubules exist with one T-tubule atop each z disc, whereas the makeup of skeletal T-tubules exist with two T-tubules astride one z disc. It

is tempting to hypothesize that different isoforms of Bin1 contribute to this classic differentiation between cardiac and skeletal muscle. Data exist that, in cardiac muscle, less cBIN1 causes widened T-tubules (Liu et al., 2020), not unlike that of failing heart muscle (Li et al., 2020, whereas in skeletal muscle less overall Bin1 but more Bin1 exon 17 leads to narrowed T-tubules (Romer et al., 2021). It would be interesting to explore in detail skeletal T-tubules in models with less cardiac Bin1, and vice versa. The therapeutic benefit of exogenous cBIN1 in failing heart muscle (Li et al., 2020) suggests that skeletal muscle may potentially benefit from exogenous Bin1 as well (Prokic et al., 2020).

A limitation of the current study by Romer et al. (2021) is the lack of rescue experiments to conclude sufficiency and causality from otherwise associated findings. For instance, does introduction of exogenous Bin1, or inhibition of isoforms that include exon 17, recover normal diameter T-tubules in R6/2 myocytes? Similarly, would exogenous JPH recover either T-tubule diameter or spacing between T-tubules and SR (Reynolds et al., 2013; van Oort et al., 2011)? While the present findings are important and significant, rescue experiments would help establish causality. It is quite possible that other candidate proteins, still yet to be identified, could be active in altered skeletal T-tubules.

The authors are to be congratulated for having identified a primary ultrastructural myotubular pathology in HD skeletal muscle which consists of abnormally narrow T-tubules. Such T-tubule changes can at least partially contribute to the loss of specific capacitance in HD muscle. Related to altered T-tubules, the authors also identified a decrease in Bin1 and JPH expression protein levels and aberrant splicing of Bin1 with an increase of a Bin1 isoform that contains exon 17. It remains to be established if the reported changes in Bin1 and JPH expression are causal of the altered skeletal T-tubules. Given the burgeoning knowledge of T-tubule organization in both skeletal and cardiac muscle, as well as the proteins that affect T-tubule membrane organization, the study by Romer et al. (2021) contributes to a better understanding of HD pathogenesis and presents new tools to understand the developmental mechanisms of T-tubules in both striated skeletal and cardiac muscle.

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