A sarcomeric protein tongue-twister: post-translation, citrullination/deimination and elimination of arginine residues

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This editorial refers to ‘Citrullination of myofilament proteins in heart failure’ by J. Fert-Bober et al., pp. 232–242.

1. Discovery of citrullination of cardiac sarcomeric proteins

There is now appreciation in the research community that post-translational modification (PTM) at the level of cardiac sarcomeres is a significant element in the control of cardiac contractility down-stream of Ca-signaling.1,2 These modifications are critical in the tuning of the heart beat to impositions of altered heart rate, pre-load, afterload, and metabolic demands. The constellation of cardiac sarcomeric PTMs that occur are related to the maintenance of a homeostatic state, and as with other regulatory mechanisms, when the PTMs do not synchronize with demands on cardiac function a progressive disorder may occur. With the emergence of remarkably sophisticated aches employing mass spectrometry,3,4 there has been a deeper understanding of the diversity and potential significance of PTMs of sarcomeric proteins beyond their well-characterized state of phosphorylation and oxidation. The list includes a multitude of potential modifications, and a newly discovered modification has been reported by Fert-Bober et al.5 This novel finding indicates that a number of critical cardiac sarcomeric proteins from control human hearts and from hearts with ischaemic disease and dilated cardiomyopathy undergo deimination or citrullination in which an Arg residue is replaced with a citrulline, resulting in an irreversible loss of a negative charge and an increase in hydrophobicity.6 Charge changes in this region may affect the ability of the unique flexible N-terminal extension cTnI to the rest of the protein.7 Charge changes in this region may affect the ability of phosphorylation of Ser23/Ser24 to desensitize myofilaments to Ca2+.8 There is also phosphorylation site at Thr31.9 Moreover, other peptides (residues 165–174 and 194–205) reside in a significantly im-
gressive C-terminal region likely to be engaged in overlap with a contiguous Tm-1. Communication of Tm-1 units along the thin filament is considered as an important aspect of the co-operative activation of the thin filament.12 The mass spectrometry analysis identified three citrullinated peptides of MyBP-C, which are located at either end of the protein in

myosin heavy chain, myosin light chains, cardiac myosin binding protein C (cMyBP-C), and each of the major thin filament proteins, actin, tropomyosin (Tm-1), cardiac troponin I (cTnI), and troponinT (cTnT). There were also peptides in Z-disc and M-band-related proteins as well as cytoskeletal proteins such as desmin, filamen, myo-

mesin, myozenin, LIM protein, and vimentin.

As examples of the relative importance of Arg residues identified as deleted by citrullination, I speculate on the functional significance of the regions demonstrating this PTM in the major sarcomeric proteins, cTnI, Tm-1, MyBP-C, and the regulatory myosin light chain (MLC2). In the case of cTnI, four peptide regions identified as citrullinated were in the N-terminal and C-terminal regions as well as near C-terminal re-
gion. The N-terminal citrullination sites were in peptides (residues 26–36 and 59–72) flanking a significant regulatory domain containing Ser42 and Ser44 in a highly charged region of cTnI. Phosphorylation of Ser42/Ser44 reduces skinned fibre tension and Ca-sensitivity.1 It seems likely that removal of the Arg charge at these peptides would affect function. Moreover, residues 26–36 are located in the region connecting the unique flexible N-terminal extension cTnI to the rest of the protein.7 Charge changes in this region may affect the ability of phosphorylation of Ser23/Ser24 to desensitize myofilaments to Ca2+.9,10 A Lys206Glu missense mutation in this region has been reported to be linked to hypertrophic cardiomyopathy.11 This loss of charge by a mutation is similar to that associated with citrullination and indicates how what may seem a minor modification is amplified and promotes dysfunction and maladaptive hypertrophy.

The Tm-1 peptide citrullinated was located at residues 236–251, which is a near C-terminal region likely to be engaged in overlap with a contiguous Tm-1. Communication of Tm-1 units along the thin filament is considered as an important aspect of the co-operative activation of the thin filament.12

The mass spectrometry analysis identified three citrullinated peptides of MyBP-C, which are located at either end of the protein in
regions not clearly understood in terms of specific function. A C-terminal peptide (residues 40–54) is located in an immunoglobulin (Ig) domain, which may interact with the thin filament or MLC2.13 The other peptides (residues 1091–1099 and 1156–1173) are also in Ig domains, which interact with titin and light meromyosin. Concepts of the role and molecular mechanisms of MyBP-C in controlling cardiac dynamics are still emerging.14,15 There is evidence that phosphorylation of MyBP-C controls cardiac dynamics and possibly sarcomere Ca-responsiveness, but the relative role of its interactions with myosin or with the thin filament remains to be resolved. Whatever the case, there is also evidence that oxidative modifications of regions of MyBP-C not generally considered to have major functional effect do induce altered sarcomere dynamics.16 Thus, long-range intra-molecular effects of modifications of MyBP-C by citrullination in peptides up- and down-stream of the well-described phosphorylation domain of MyBP-C may have functional consequences.

Although not discussed by the authors, the changes in the N-terminal region of myosin light chain 2 may also be significant in that citrullination occurs near the phosphorylation site and in the Ca-binding domain. Functional significance of these regions has been discussed previously.17–19 It is apparent that the phosphorylation and Ca-binding to MLC2 maintains normal cardiac function by modifying the radial movements of cross-bridges from the thick filament.20 It will be of interest to know whether the effect of citrullination modifies the homeostatic effects of MLC2 phosphorylation.

2. There is a long road ahead in clarifying the functional significance of sarcomere protein citrullination

In an approach to provide evidence that the citrullination is indeed functionally significant, Fert-Bober et al.5 incubated sarcomeric proteins and detergent-extracted cardiac myocytes with activated PAD2 and used read-outs of ATPase rate, protein–protein interactions, and force generation. As with many new insights into molecular mechanisms, the results of these experiments raise many more questions than they answer. Studies investigating the effect of incubations with active PAD2 on ATPase activity demonstrated an inhibition with citrullination in heavy meromyosin (HMM) alone and with HMM in the presence of actin or actin-Tm-1. Citrullination promoted binding of Tm-1 to actin, but had no effect on the binding of cTnl to actin. The most revealing studies of the integrated effects of citrullination are those carried out with single detergent-extracted ventricular myocytes incubated with vehicle or with active PAD2. There was a modest effect on the Ca-sensitivity of sarcomeric tension following incubation with PAD2, which fits generally with the finding that citrullination of Tm-1 induced increased binding of Tm-1 to actin filaments. There was no effect of myocyte citrullination on the steepness of the Ca-tension relation, but further studies are likely to explore this possibility more completely.

The mechanical data in the detergent-extracted myocytes are difficult to relate to the ATPase measurements inasmuch as there were no measures of tension cost or pre-steady state mechanical function reflecting cross-bridge kinetics. Measurements in the future determining cross-kinetics as well ATP hydrolysis in skinned fibres will reveal whether the findings from the ATPase measurements are relevant to altered function in the intact sarcomeres of myocytes. Despite the modifications in cTnl in functionally significant regions by citrullination, in the data reported by Fert-Bober et al., there was no effect of cTnl citrullination on the binding of cTnl to actin-Tm-1. It would be important to further explore the possible functional role of cTnl citrullination using a more complete set of assays.

The data are not definitive with regard to the effect of citrullination on the response of human sarcomeric proteins to Ca2+. It seems likely that peptides identified in samples from end-stage heart failure patients are different from those obtained with in vitro studies with mouse myocytes. Thus, the effects of citrullination in vitro may differ substantially from the in situ modifications by PAD activated in the pathological cellular environment. Moreover, there is a high probability of interactions among the various PTMs in the human samples (e.g. oxidative and phosphorylation modifications). The cardiac myocytes are from healthy mice, likely to have differences in sarcomeric PTMs differing from those in the samples from humans with cardiac disorders. It will be important for future investigations to use human cardiac sarcomeres in an attempt to recapitulate the citrullination identified with end-stage heart samples.

3. RA as a disease of the cardiac sarcomere

Despite the difficulties in understanding the functional role of citrullination from studies done so far, an exciting aspect of the findings of Fert-Bober and colleagues5,6,21 is the demonstration of a potential role of modified cardiac sarcomeres in RA. Their data add to the complexity of understanding control of cardiac function, but also add some clarity to the possible reasons why patients with RA have a higher incidence of morbidity and mortality from cardiovascular dysfunction. It has been known since the 19th century that there is an increased incidence of cardiovascular disease in patients with RA. More recent studies comparing RA patients with non-RA patients reported the presence of systolic and diastolic dysfunction that appears to manifest in RA patients independently of coronary artery disease.22 Histological studies by Giles et al.21 with archived autopsy tissue concluded that heart samples from patients with RA had significantly higher citrullination than other disease states, but only in interstitial regions. These data indicated that the heart failure associated with RA involves working heart muscle per se. Earlier studies attributed the heart failure largely to inflammation of the pericardium.23 Moreover, knowledge that protein citrullination is an important mechanism in the pathology of RA has also been recognized for nearly 20 years with the report that autoantibodies that reacted with the peptides containing citrulline were specifically found in the sera of patients with RA. Fert-Bober et al. provide an advance in understanding in this field by their identification that citrullination is likely to occur in the sarcomeres of working cardiac myocytes and to have a negative inotropic effect. The finding offers the potential for extensive further investigations attempting to sort out the complex modifications that occur in sarcomeric proteins in RA and various diseases and what they mean.

4. The future

The major challenge to taking this field forward is embodied in Supplemental material online, Figure S2 of Fert-Bober et al.3 In this figure, the authors have incorporated the changes in citrullination into the human STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database of protein interactions in humans. On the surface, these are
powerful interpretations of the data, but in the trenches of the labora-
tory trying to improve heart disease, e.g. in victims of arthritis, the in-
terpretation is confusing and disturbing with regard to the hope of
designing rational translational experiments.

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