LETTER

Reply from Chun Y. Seow

Behind the controversy: the elusive contractile unit of smooth muscle

Imagine if the sarcomeric structure were unbeknownst to us, our knowledge of the contraction mechanism of striated muscle would be in a woefully incomplete state. Our knowledge about smooth muscle contraction, unfortunately, is in such a state, because the structure of the contractile unit (sarcomere equivalent in smooth muscle) is largely unknown. Today we know more about the molecular mechanism of actomyosin interaction than we do about the mechanism by which the interaction is translated into cell motility, simply because of our lack of understanding of the structure of the filamentous lattice within which the interaction occurs. Myosin filament is an integral part of the contractile unit. Many would agree that knowing the myosin filament length or length distribution in smooth muscle is an important step towards elucidating the structure of the contractile unit. We therefore very much appreciate the comments by Dr Somlyo regarding our report (Liu *et al.* 2013) on myosin filament length in smooth muscle, and hope that this would rekindle interest in this important area of research. In the report we compared our measurements of filament length from electron micrographs of serial transverse cell sections with the same measurements made by Ashton *et al.* (1975) using a similar method. However, we overlooked their measurements from longitudinal sections that show long continuous myosin filaments with a mean length of 2.2 μm. Results from the two studies are fundamentally different because we (Liu *et al.* 2013) did not find a normal distribution for myosin filament lengths from which a mean length could be obtained; instead we found a length distribution resembling an exponential decay (that is, the longer the filaments, the less frequently they were observed). We did encounter filaments with length 2.2 μm or longer, but the significance of our finding lies in the much greater number of shorter filaments that we also encountered (Fig. 5*A* in Liu *et al.* 2013). It should be pointed out that other investigators have measured myosin filament length in smooth muscle and obtained different results. For example, filament lengths ranging from 1.6 to 8 μm have been reported by Small and colleagues (Small, 1977; Small *et al.* 1990). The controversy is therefore very much alive and there is no clear explanation for the discrepancy. Behind the controversy over the myosin filament length there is a bigger controversy over whether the contractile apparatus of smooth muscle resembles that of striated muscle or non-muscle motile cells. If a uniform myosin filament length exists in smooth muscle, it would lend support to the hypothesis that the contraction mechanism in smooth muscle is fundamentally similar to that in striated muscle. However, if non-uniform myosin filaments exist in smooth muscle, the possibility for smooth muscle possessing a fundamentally different contraction mechanism from that of striated muscle needs to be considered. If the contraction mechanisms of striated muscle and non-muscle motile cells represent two extremes of a spectrum, it is possible that the contraction mechanisms for different types of smooth muscle fall on different points within that spectrum. An important question is, do different types of smooth muscle possess fundamentally different contraction mechanisms? Or is the difference just quantitative in nature?

In the studies of Liu *et al.* (2013) we also measured the myosin filament length in longitudinal sections. The purpose of making those measurements was to demonstrate that in thin longitudinal sections myosin filament length could be underestimated, especially for long filaments. The results were not surprising; the measured filament lengths from longitudinal sections were consistently shorter than those measured from serial sections, except for the very short filaments (Fig. 6*B* in Liu *et al.* 2013). We have pointed out that this is likely because not all myosin filaments are perfectly in parallel with the surface of the longitudinal section, and the 'likelihood for the filament exiting the plane of the longitudinal section increases with increasing length of the filament'. However, like that in the measurements from serial sections, the filament length distribution in longitudinal sections also did not show a normal or semi-normal distribution.

In an earlier study (Herrera *et al.* 2005) we showed examples of myosin filaments 1.8–2.2 μm in length in pig trachealis. No systematic measurement of myosin filament length was carried out in that study and the purpose of showing those images was to provide evidence that a contractile unit structure such as that proposed by Hodgkinson *et al.* (1995) could exist. We further pointed out in that study that 'Although the existence of contractile units in smooth muscle has a sound functional basis, there is no direct structural evidence. So far, we have relied on a few 'glimpses' of the ultrastructure for clues on how the contractile machinery of smooth muscle may be constructed'. We further cautioned that 'non-uniform filament lengths should also be kept in mind' (Herrera *et al.* 2005). Our belief is, although species and tissue type differences may account for some quantitative difference in the measured myosin filament length, such as those found between airway and arterial smooth muscles in the studies of Liu *et al.* (2013), it is less likely that a qualitative difference (such as that between uniform and non-uniform length distribution) exists between different types of smooth muscle representing fundamentally different mechanisms regulating myosin filament formation.

Most *in vitro* studies of smooth muscle myosin filament formation were carried out at unphysiologically low ionic strengths. We have observed self-assembly of purified airway smooth muscle myosin into filaments at an ionic strength of 88 mM (Ip *et al.* 2007). At the physiological ionic strength of ~200 mM and 5 mM [MgATP], virtually no filament could be seen. The existence of myosin filaments in intact smooth muscle cells is in many ways a mystery, although the filament formation could be facilitated by the presence of actin filament lattice and/or the presence of myosin binding proteins on the thin filaments such as caldesmon (Seow, 2005). Our interest in measuring changes in myosin filament length was sparked by the phenomenon of myosin evanescence – that is, changes in filament mass under different conditions (Gillies *et al.* 1988; Godfraind-De Becker & Gillis, 1988; Watanabe *et al.* 1993; Xu *et al.* 1997; Herrera *et al.* 2002; Qi *et al.* 2002; Kuo *et al.* 2003; Smolensky *et al.* 2005).

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The quantifiable myosin evanescence in many types of smooth muscle suggests that the fixed sarcomeric type of contractile apparatus seen in striated muscle may not exist in smooth muscle.

Another important component of the contractile unit is the thin filaments. There is no consensus on how long the thin filaments are in smooth muscle; the estimates range from 1.35 (Drew & Murphy, 1997) to 4.5 μm (Small *et al.* 1990). Adding to the complexity is the overwhelming evidence that actin filaments are not static; both their length and attachment to other cell structures are dynamic and rigorously regulated (Mehta & Gunst, 1999; Gunst & Fredberg, 2003; Herrera *et al.* 2004; Tejani *et al.* 2011; Walsh & Cole, 2013). The degree of malleability in the cytoskeleton of smooth muscle is so remarkable that 'fluidization' and 'solidification' are used to describe the muscle behaviour (Krishnan *et al.* 2009; Chen *et al.* 2010). This is further evidence suggesting that the static sarcomeric structure seen in striated muscle is not likely to be present in smooth muscle.

We have found recently that in the presence of a Rho-kinase inhibitor the observable myosin filament mass in airway smooth muscle is greatly reduced compared with that in a force-matched control (Lan *et al.* 2014), suggesting that Rho-kinase is involved in the regulation of myosin filament formation or redistribution of myosin filament lengths. The observation that smooth muscle can generate the same amount of force with very different masses of observable myosin filaments is intriguing. However, recognizing the limitation of electron microscopy in identifying short filaments one could hypothesize that inhibition of Rho-kinase could result in a shift of myosin filament distribution to shorter lengths and thus more filaments become 'invisible'. These short filaments could still participate in the process of force generation because each side-polar filament, regardless of its length, acts as a ratchet to pull the thin filaments in opposite directions and contribute to force generation, as postulated by Lan *et al.* (2014, Fig. 10). In this model, under static conditions (isometric contraction) a muscle will generate the same amount of force regardless of the distribution pattern of the myosin filaments, as long as the same number of myosin dimers participates in the force generation. However, under dynamic conditions such as when length oscillations are applied to a contracting muscle, longer myosin filaments may allow a muscle to maintain force better than a muscle with shorter filaments, assuming that shorter filaments such as dimers could be more easily dislodged from the thin filament lattice during large oscillations when the ratchets are not overlapped by thin filaments on both sides. Evidence supporting this hypothesis is provided by the study of Lan *et al.* (2014), which shows that muscles with less (observable) myosin filament mass are less able to maintain force during length oscillation, while in isometric contraction their ability to generate force is not affected. Much more rigorous testing of the model (Lan *et al.* 2014) is needed because it is based on assumptions that are obviously biased towards the belief that the structures of the contractile apparatus and cytoskeleton in smooth muscle are highly labile and malleable.

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Additional information

Competing interests

The author declares no actual or potential conflicts of interest.