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## Biophysical basis of airway smooth muscle contraction and hyperresponsiveness in asthma

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### 1.1 Introduction

It is self-evident that acute narrowing of the asthmatic airways and shortening of the airway smooth muscle are inextricably linked. Nonetheless, it was many years ago that research on the asthmatic airways and research on the biophysics of airway smooth muscle had a parting of the ways (Seow and Fredberg, 2001). The study of smooth muscle biophysics took on a life of its own and pursued a deeply reductionist agenda, one that became focused to a large extent on myosin II and regulation of the actomyosin cycling rate. The study of airway biology pursued a reductionist agenda as well, but one that became focused less and less on contractile functions of muscle and instead emphasized immune responses, inflammatory cells and mediators, and, to the extent that smooth muscle remained

of interest, that interest centred mainly on its synthetic, proliferative and migratory functions (Amrani and Panettieri, 2003; Black and Johnson, 1996; 2000; Black *et al.*, 2001; Holgate *et al.*, 2003; Kelleher *et al.*, 1995; Zhu *et al.*, 2001). Inflammatory remodelling of the airway wall was also recognized as being a key event in the asthmatic diathesis (Dulin *et al.*, 2003; Homer and Elias, 2000; James *et al.*, 1989; McParland *et al.*, 2003; Moreno *et al.*, 1986; Paré *et al.*, 1991; Wang *et al.*, 2003).

To better understand the impact of inflammatory remodelling processes upon smooth muscle shortening and acute airway narrowing, computational models of ever increasing sophistication were formulated, but, remarkably, the muscle compartment of these models remained at a relatively primitive level, being represented by nothing more than the classical relationship of active isometric force versus muscle length (Lambert and Paré, 1997; Lambert *et al.*, 1993; Macklem, 1987; 1989; 1990; 1996; Wiggs *et al.*, 1992). As discussed below, this description is now considered to be problematic because the very existence of a well-defined static force-length relationship has of late been called into question, as has the classical notion that the muscle possesses a well-defined optimal length. Rather, other factors intrinsic to the airway smooth muscle cell, especially muscle dynamics and mechanical plasticity, as well as unanticipated interactions between the muscle and its load, are now understood to be major factors affecting the ability of smooth muscle to narrow the airways (An *et al.*, 2007; Fredberg, 2000a; Fredberg *et al.*, 1999; Pratusевич *et al.*, 1995; Seow and Fredberg, 2001; Seow and Stephens, 1988; Seow *et al.*, 2000).

The topics addressed in this chapter are intended to highlight recent discoveries that bring airway biology and smooth muscle biophysics into the same arena once again. Here we do not provide an exhaustive review of the literature, but rather emphasize key biophysical properties of airway smooth muscle as they relate to excessive airway narrowing in asthma. This is appropriate because, in the end, if airway inflammation did not cause airway narrowing, asthma might be a tolerable disease. But asthma is not a tolerable disease. In order to understand the multifaceted problem of airway hyperresponsiveness in asthma, therefore, an integrative understanding that brings together a diversity of factors will be essential.

## 1.2 Airway hyperresponsiveness

It was recognized quite early that the lung is an irritable organ and that stimulation of its contractile machinery in an animal with an open chest can cause an increase

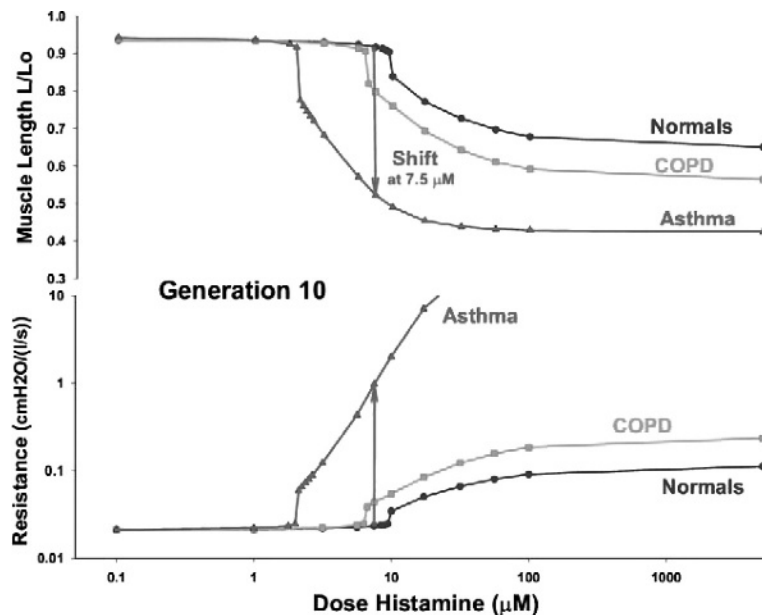
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in lung recoil, an expelling of air, a rise in intratracheal pressure, and an increase in airways resistance (Colebatch *et al.*, 1966; Dixon and Brodie, 1903; Mead, 1973; Otis, 1983). The fraction of the tissue volume that is attributable to contractile machinery is comparable for airways, alveolated ducts and blood vessels in the lung parenchyma (Oldmixon *et al.*, 2001); the lung parenchyma, like the airways, is a contractile tissue (Colebatch and Mitchell, 1971; Dolhnikoff *et al.*, 1998; Fredberg *et al.*, 1993; Ludwig *et al.*, 1987; 1988). Although airway smooth muscle was first described in 1804 by Franz Daniel Reisseisen (as related by Otis (1983)) and its functional properties first considered by Einthoven (1892) and Dixon and Brodie (1903), until the second half of the last century this muscle embedded in the airways was not regarded as being a tissue of any particular significance in respiratory mechanics (Otis, 1983). A notable exception in that regard was Henry Hyde Salter, who, in 1859, was well aware of the 'spastic' nature of airway smooth muscle and its potential role in asthma (Salter, 1868). The airway smooth muscle is now recognized as being the major end-effector of acute airway narrowing in asthma (Lambert and Paré, 1997; Macklem, 1996). There is also widespread agreement that shortening of the airway smooth muscle cell is the proximal cause of excessive airway narrowing during an asthmatic attack (Dulin *et al.*, 2003), and swelling of airway wall compartments and plugging by airway liquid or mucus are important amplifying factors (Lambert and Paré, 1997; Yager *et al.*, 1989). It remains unclear, however, why in asthma the muscle can shorten excessively.

'Airway hyperresponsiveness' is the term used to describe airways that narrow too easily and too much in response to challenge with nonspecific contractile agonists (Woolcock and Peat, 1989). Typically, a graph of airways resistance versus dose is sigmoid in shape (Figure 1.1); the response shows a plateau at high levels of contractile stimulus. The existence of the plateau, in general, is interpreted to mean that the airway smooth muscle is activated maximally and, thereby, has shortened as much as it can against a given elastic load. Once on the plateau, therefore, any further increase in stimulus can produce no additional active force, muscle shortening, or airway resistance.

To say that airways narrow too easily, on the one hand, means that the graph of airways resistance versus dose of a nonspecific contractile stimulus is shifted to the left along the dose axis, and that airways respond appreciably to levels of stimulus at which the healthy individual would be unresponsive; this phenomenon is called hypersensitivity. To say that airways narrow too much, on the other hand, means that the level of the plateau response is elevated, or that the plateau is abolished altogether, regardless of the position of the curve along the dose axis; this phenomenon is called hyperreactivity. As distinct from hypersensitivity, it is



**Figure 1.1** Computation of airway hyperresponsiveness in asthma. A computational result showing airway length (top) and airway resistance (bottom) as a function of agonist concentration for a 10th-generation airway (Mijailovich, 2003). The cases shown depict airways from a normal, an asthmatic and a COPD (Chronic obstructive pulmonary disease) lung. In this computation, the effects of tidal breathing and deep inspirations (6/min) upon myosin binding dynamics are taken into account explicitly (Mijailovich, 2003). As explained in the text, such an airway exhibits both hyperreactivity and hypersensitivity. (Reproduced courtesy of the American Journal of Respiratory and Critical Care Medicine 167, A183.)

this ability of the airways to narrow excessively, with an elevated or abolished plateau, that accounts for the morbidity and mortality associated with asthma (Sterk and Bel, 1989).

It has long been thought that the factors that cause hypersensitivity versus hyperreactivity are distinct, the former being associated with receptor complement and downstream signalling events but the latter being associated with purely mechanical factors, including the contractile apparatus, the cytoskeleton (CSK), and the mechanical load against which the muscle shortens (Armour *et al.*, 1984; Lambert and Paré, 1997; Macklem, 1996; Wiggs *et al.*, 1992). Macklem has pointed out that, once the muscle has become maximally activated, it is the active force and the load that become all-important, and the plateau response becomes essentially uncoupled from underlying biochemistry, signalling and cell biology

(Macklem, 1987; 1990; 1996). However, as described below, there is reason to think that these distinctions may not be as clear as once believed.

Although asthma is usually defined as an inflammatory disease, the link between the immunological phenotype and the resulting mechanical phenotype associated with disease presentation, including airway hyperresponsiveness, remains unclear; indeed, it is now established that airway hyperresponsiveness can be uncoupled from airway inflammation (Bryan *et al.*, 2000; Crimi *et al.*, 1998; Holloway *et al.*, 1999; Leckie *et al.*, 2000). It remains equally unclear whether airway hyperresponsiveness is due to fundamental changes within the smooth muscle itself, as might be caused by inflammatory mediators, chemokines and cytokines (Fernandes *et al.*, 2003), or due to changes external to the muscle, such as a reduced mechanical load against which the smooth muscle contracts. Still another possibility supported by recent evidence is that there is an interaction of the two wherein the contractile machinery within the smooth muscle cell adapts in response to a change in its mechanical microenvironment (Dulin *et al.*, 2003; Fredberg *et al.*, 1999; Lakser *et al.*, 2002; Pratusевич *et al.*, 1995; Seow and Fredberg, 2001; Seow *et al.*, 2000; Wang *et al.*, 2001). Moreover, Tschumperlin *et al.* (2002; 2003) have provided evidence that bronchospasm can lead to mechanically induced pro-inflammatory signalling events in the airway epithelium, in which case airway inflammation may cause bronchospasm, but bronchospasm in turn may amplify or even activate specific inflammatory pathways.

In the balance of this chapter, we address the classical picture of smooth muscle behaviour and then go on to describe what we know about nonclassical behaviour in a dynamic setting and, in particular, the ability of the muscle cell to adapt rapidly to changes in its mechanical microenvironment. We do not address the increasing evidence that now suggests that cytokines such as interleukin (IL)-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$  augment responses to bronchoconstrictor agonists while attenuating the bronchodilation that can be effected by hormones and paracrine agents such as epinephrine and PGE<sub>2</sub> (Shore *et al.*, 1997). Such cytokines, along with growth factors and other inflammatory mediators, also result in smooth muscle hyperplasia, at least in culture systems (Kelleher *et al.*, 1995). In culture, extracellular matrix proteins have been shown not only to regulate synthetic (Chan *et al.*, 2006; Peng *et al.*, 2005), proliferative (Freyer *et al.*, 2001; Hirst *et al.*, 2000; Nguyen *et al.*, 2005) and migratory (Parameswaran *et al.*, 2004) functions of the airway smooth muscle cell, but also to modulate the protein expressions and biochemical pathways that are implicated in muscle maturation and contraction (Freyer *et al.*, 2004; Halayko and Solway, 2001; Halayko *et al.*, 1999; Hirst *et al.*, 2000; Tran *et al.*, 2006). Whether airway inflammation and matrix remodelling

in the asthmatic airways can result in a hypercontractile phenotype of the airway smooth muscle cell remains to be established.

### 1.3 Classical behaviour of airway smooth muscle and the balance of static forces

The microstructure of striated muscle is highly ordered, whereas there is abundant evidence in the literature demonstrating that the cytoskeletal microstructure of smooth muscle is quite disordered (Small, 1995; Small and Gimona, 1998); it is, after all, its amorphous structure that gives 'smooth' muscle its name. Moreover, the airway smooth muscle CSK is in a continuous state of remodelling, a point to which we return below. Despite these differences, it has been widely presumed that to a first approximation Huxley's sliding-filament model of muscle contraction (Huxley, 1957) describes the function of both smooth and striated muscle (Murphy, 1988; 1994; Mijailovich *et al.*, 2000). For many of the biophysical phenomena observed in airway smooth muscle, such as active force generation and shortening velocity, Huxley's model represents a useful tool for thought (Huxley, 1957; Mijailovich *et al.*, 2000), while for others, such as mechanical plasticity, it does not.

As in the case of striated muscle contraction, the principal biophysical parameters that characterize smooth muscle contraction include the maximum active isometric force (or stress, which is simply the force carried per unit area), the length at which the muscle can attain that maximal force (i.e., the optimum length ( $L_0$ )), and the shortening capacity of the muscle. The sliding-filament model of Huxley is the starting point for understanding each of these phenomena. As described by Huxley (1957), isometric force, as well as muscle stiffness, is proportional to the number of actomyosin cross links per unit volume. This is true because, assuming rigid filaments, all bridges within a given contractile unit must act mechanically in parallel, with their displacements being identical and their forces being additive. The maximum active stress supported by smooth versus striated muscle is approximately the same and is of the order  $10^5$  Pa. In striated muscle,  $L_0$  is attributed to the extent of overlap between the myosin filament and the actin filament,  $L_0$  corresponding to a maximum number of myosin heads finding themselves within the striking distance of an available binding site on the actin filament, and the maximum capacity of the muscle to shorten being limited by the collision of the myosin filament end with the z-disc. Smooth muscle possesses no structure comparable to the z-disc, however, although actin filaments

terminate in dense bodies, which might come into play in limiting muscle shortening. Whereas unloaded striated muscle can shorten perhaps 20 per cent from its optimum length, unloaded smooth muscle can shorten as much as 70 per cent (Stephens, 1987; Stephens and Seow, 1993; Uvelius, 1976). Several physical factors may come into play to limit the capacity for unloaded shortening of smooth muscle. Small (1995) has shown that actin filaments of the contractile apparatus connect to the CSK at cytoplasmic dense bodies and with the longitudinal rib-like arrays of dense plaques of the membrane skeleton that couple to the extracellular matrix. Moreover, the side-polar configuration of the myosin filament (Tonino *et al.*, 2002; Xu *et al.*, 1996) is likely to be involved. Still other factors coming into play include length-dependent activation (An and Hai, 1999; 2000; Mehta *et al.*, 1996; Youn *et al.*, 1998), length-dependent rearrangements of the CSK and contractile machinery (Gunst *et al.*, 1995; Pratusевич *et al.*, 1995), and length-dependent internal loads (Stephens and Kromer, 1971; Stephens and Seow, 1993; Warshaw *et al.*, 1988).

What are the extramuscular factors that act to limit airway smooth muscle shortening? The basic notion, of course, is that muscle shortening stops when the total force generated by the muscle comes into a static balance with the load against which the muscle has shortened, both of which vary with muscle length. The factors setting the load include the elasticity of the airway wall, elastic tethering forces conferred by the surrounding lung parenchyma, active tethering forces conferred by contractile cells in the lung parenchyma (Nagase *et al.*, 1994; Romero and Ludwig, 1991), mechanical coupling of the airway to the parenchyma by the peribronchial adventitia, and buckling of the airway epithelium and submucosa (Ding *et al.*, 1987; Robatto *et al.*, 1992; Wiggs *et al.*, 1992). In addition, the airway smooth muscle itself is a syncytium comprised mostly of smooth muscle cells, aligned roughly along the axis of muscle shortening, and held together by an intercellular connective tissue network. In order to conserve volume, as the muscle shortens, it must also thicken. And as the muscle shortens and thickens, the intercellular connective tissue network must distort accordingly. Meiss (1999) has shown that at the extremes of muscle shortening it may be the loads associated with radial expansion (relative to the axis of muscle shortening) of the intercellular connective tissue network that limit the ability of the muscle to shorten further.

In the healthy, intact dog, airway smooth muscle possesses sufficient force-generating capacity to close all airways (Brown and Mitzner, 1998; Warner and Gunst, 1992). This fact may at first seem to be unremarkable, but it is not easily reconciled with the observation that when healthy animals or people are challenged with inhaled contractile agonists in concentrations thought to be sufficient

to activate the muscle maximally, the resulting airway narrowing is limited in extent, and that limit falls far short of airway closure (Moore *et al.*, 1997; 1998). Breathing remains unaccountably easy. Indeed, it is this lightness of breathing in the healthy challenged lung, rather than the labored breathing that is characteristic of the asthmatic lung, that in many ways presents the greater challenge to our understanding of the determinants of acute airway narrowing (Fredberg and Shore, 1999). Brown and Mitzner (1998) have suggested that the plateau of the dose-response curve reflects uneven or limited aerosol delivery to the airways. Another possibility, however, is that some mechanisms act to limit the extent of muscle shortening in the healthy, breathing lung, whereas these mechanisms become compromised in the asthmatic lung. It has been suspected that the impairment of that salutary mechanism, if it could only be understood, might help to unlock some of the secrets surrounding excessive airway narrowing in asthma, as well as the morbidity and mortality associated with that disease (Fish *et al.*, 1981; Lim *et al.*, 1987; Nadel and Tierney, 1961; Skloot *et al.*, 1995). This brings us to muscle dynamics and the factors that could account for airway hyperresponsiveness in asthma.

#### 1.4 Shortening velocity and other manifestations of muscle dynamics

The oldest and certainly the simplest explanation of airway hyperresponsiveness would be that muscle from the asthmatic airways is stronger than muscle from the healthy airways, but evidence in support of that hypothesis remains equivocal (Black and Johnson, 1996; 2000; De Jongste *et al.*, 1987; Solway and Fredberg, 1997). Indeed, a number of earlier studies, in which tissues were obtained post-mortem or surgically, have reported normal contractility (Bai, 1990; Bjorck *et al.*, 1992) and even hypocontractility (Goldie *et al.*, 1986; Whicker *et al.*, 1988) of muscle from the asthmatic airways. Accordingly, studies from the laboratory of Stephens and colleagues (Antonissen *et al.*, 1979; Fan *et al.*, 1997; Jiang *et al.*, 1992; Ma *et al.*, 2002; Seow and Stephens, 1988) have emphasized that the force-generation capacity of allergen-sensitized airway smooth muscle of the dog, or of human asthmatic muscle, is no different from that of control muscle. As a result, the search for an explanation turned to other factors, and several alternative hypotheses have been advanced. These fall into three broad classes, each of which is consistent with remodelling events induced by the inflammatory microenvironment, and they include an increase of muscle mass (Johnson *et al.*, 2001;

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Lambert *et al.*, 1993; Thomson *et al.*, 1996; Wiggs *et al.*, 1992), a decrease of the static load against which the muscle shortens (Ding *et al.*, 1987; Macklem, 1996; Wiggs *et al.*, 1992; 1997), and a decrease of the fluctuating load that perturbs myosin binding during breathing (Fredberg, 1998; 2000a; 2000b; Fredberg *et al.*, 1999; Mijailovich *et al.*, 2000). Taken together, these hypotheses are attractive because they suggest a variety of mechanisms by which airway smooth muscle can shorten excessively even while the isometric force-generating capacity of the muscle remains essentially unchanged.

Aside from changes in the static load and/or the dynamic load, however, a consistent association has been noted between airway hyperresponsiveness and unloaded shortening velocity of the muscle (Antonissen *et al.*, 1979; Duguet *et al.*, 2000; Fan *et al.*, 1997; Ma *et al.*, 2002; Wang *et al.*, 1997). This association suggests that the problem with airway smooth muscle in asthma may be that it is too fast rather than too strong. But how shortening velocity – a dynamic property of the muscle – might cause excessive airway narrowing – a parameter that was thought to be determined by a balance of static forces – remains unclear. To account for increased shortening capacity of unloaded cells, Stephens and colleagues have reasoned that upon activation virtually all muscle shortening is completed within the first few seconds (Ma *et al.*, 2002). As such, the faster the muscle can shorten within this limited time window, the more it will shorten. However, in isotonic loading conditions at physiological levels of load, muscle shortening is indeed most rapid at the very beginning of the contraction, but appreciable shortening continues for at least 10 min after the onset of the contractile stimulus (Fredberg *et al.*, 1999). An alternative hypothesis to explain why intrinsically faster muscle might shorten more comes from consideration of the temporal fluctuations of the muscle load that are attributable to the action of spontaneous breathing (Fredberg *et al.*, 1997; 1999; Solway and Fredberg, 1997). Load fluctuations that are attendant on spontaneous breathing are the most potent of all known bronchodilating agencies (Gump *et al.*, 2001; Shen *et al.*, 1997). Among many possible effects, these load fluctuations perturb the binding of myosin to actin, causing the myosin head to detach from actin much sooner than it would have during an isometric contraction. But the faster the myosin cycling (i.e., the faster the muscle), the more difficult it is for imposed load fluctuations to perturb the actomyosin interaction. This is because the faster the intrinsic rate of cycling, the faster will a bridge, once detached, reattach and contribute once again to active force and stiffness.

Why is muscle from the allergen-sensitized animal or asthmatic subject faster? For technical reasons, in their study on the single airway smooth muscle cell freshly isolated from bronchial biopsies obtained from an asthmatic subject,

Ma *et al.* 2002) did not measure protein expression levels of myosin light-chain kinase (MLCK), but their finding of increased content of message strongly implicates MLCK. Although regulation of myosin phosphorylation is a complex process with multiple kinases and phosphatases, this finding substantially narrows the search for the culprit that may account for the mechanical changes observed in these cells. Moreover, these studies seem to rule out changes in the distribution of myosin heavy-chain isoforms; content and isoform distributions of message from asthmatic cells showed the presence of smooth muscle myosin heavy-chain A (SM-A), but not SM-B, the latter of which contains a seven-amino-acid insert that is typical of phasic rather than tonic smooth muscle, and is by far the faster of the two isoforms (Lauzon *et al.*, 1998; Murphy *et al.*, 1997).

Using laser capture microdissection of airway smooth muscle from bronchial biopsies obtained from normal versus mild-to-moderate asthmatics, Woodruff *et al.*, 2004) also found no differences in the expressions profile of a panel of genes that are often considered markers of hypercontractile phenotype (including MLCK, however) but did detect a nearly twofold increase in the number of airway smooth muscle cells in the asthmatics. Although the source of the increased cell number (increased proliferation, decreased apoptosis, and/or increased migration) remains unclear (Hirst *et al.*, 2004; Johnson *et al.*, 2001; Lazaar and Panettieri, 2005; Madison, 2003; Woodruff *et al.*, 2004; Zacour and Martin, 1996), increased muscle mass alone is sufficient to predispose to airway hyperresponsiveness in asthma (James *et al.*, 1989; Lambert *et al.*, 1993; Moreno *et al.*, 1986). The question of whether muscle mass (quantity) and muscle contractility (quality) might covary remains to be elucidated, however. For example, it is likely that the airway smooth muscle cell in the proliferative/synthetic/maturational state might be less contractile than similar cells differentiated into a fully contractile state – an effect that would be compensatory – but no mechanical data are available to support that possibility.

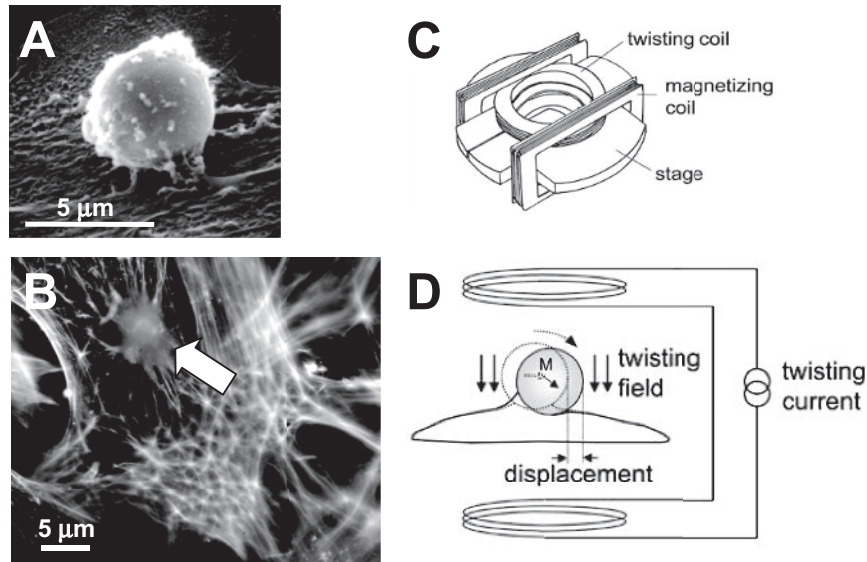
## 1.5 Biophysical characterization of airway smooth muscle: bronchospasm in culture?

With recent technological advances, such as atomic force microscopy (Alcaraz *et al.*, 2003; Smith *et al.*, 2005), two-point and laser-tracking microrheology (Van Citters *et al.*, 2006; Yamada *et al.*, 2000), magnetic tweezers (Bausch *et al.*, 1998; 1999), and traction microscopy (Butler *et al.*, 2002; Tolic-Norrelykke *et al.*, 2002), a single living cell in culture can now be characterized biophysically. While the use of cultured cells has certain limitations, they do offer the advantage

that, when passaged in culture, airway smooth muscle cells retain functional responses to a wide panel of agonists and signalling pathways that are implicated in asthma (Halayko *et al.*, 1999; Hubmayr *et al.*, 1996; Panettieri *et al.*, 1989; Shore *et al.*, 1997; Tao *et al.*, 1999; 2003; Tolloczko *et al.*, 1995). In our laboratories, to probe deeper into the mechanical properties of the airway smooth muscle cell, we use a technology that has its roots in an early contribution of Francis H. C. Crick.

Before his well-known work on the double helical structure of deoxyribonucleic acid (DNA) (Watson and Crick, 1953a; 1953b), Crick measured the viscosity and elasticity of the medium inside cells by observing internalized magnetic particles and how they rotate in reaction to an applied magnetic field (Crick and Hughes, 1950). Extending this approach, Valberg and his colleagues studied populations of particles internalized into populations of cells, and measured induced bead rotations by remote sensing, namely, by means of changes in the horizontal projection of the remanent magnetic field produced by the magnetized particles as they rotate (Valberg, 1984; Valberg and Feldman, 1987). In a major step forward, we subsequently adapted this technique still further (Fabry *et al.*, 2001; Wang *et al.*, 1993) by using ligand-coated, ferrimagnetic microbeads – not internalized as before – but rather bound to the CSK via membrane-spanning integrin receptors. And more recently still, we showed that changes in cell stiffness measured in this way correlate well with stiffness changes in the same cells measured by atomic force microscopy (Alcaraz *et al.*, 2003) and with force changes measured with traction microscopy (Wang *et al.*, 2002). This method is now known as magnetic twisting cytometry (MTC), and it has evolved into a useful tool to probe the mechanical properties of a variety of cell types, both cultured and freshly isolated, through different receptor systems, and with a variety of experimental interventions (Deng *et al.*, 2006; Fabry *et al.*, 2001; Laudadio *et al.*, 2005; Puig-de-Morales *et al.*, 2004).

The principle of MTC is straightforward (Figure 1.2). A ferrimagnetic microbead (4.5  $\mu\text{m}$  in diameter) is coated with a synthetic peptide containing the sequence Arg–Gly–Asp (RGD) and is then allowed to bind to the cell. Such an RGD-coated bead binds avidly to cell-surface integrin receptors (Wang *et al.*, 1993), forms focal adhesions (Matthews *et al.*, 2004), and becomes well integrated into the cytoskeletal scaffold (Maksym *et al.*, 2000): it displays tight functional coupling to stress-bearing cytoskeletal structures and the contractile apparatus (An *et al.*, 2002; Hu *et al.*, 2003). By imposition of a uniform magnetic field upon the magnetized bead, a small torque is applied and resulting bead motions deform structures deep in the cell interior (Hu *et al.*, 2003). Such *forced* bead motions are impeded by mechanical stresses developed within the cell body, and the ratio of

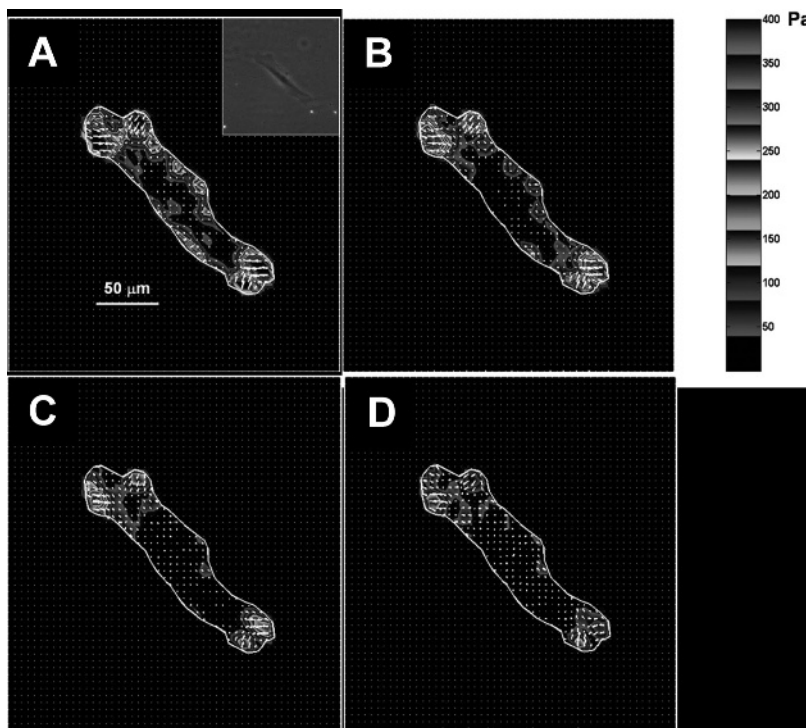


**Figure 1.2** Optical magnetic twisting cytometry (OMTC). (A) An RGD-coated bead ( $4.5 \mu\text{m}$  in diameter) binds to the surface of the adherent cell. (B) Such bead (white arrow) becomes well-integrated into underlying actin lattice (phalloidin staining). (C) The bead is magnetized horizontally (parallel to the surface on which cells are plated) and then twisted in a vertically aligned homogenous magnetic field that is varying sinusoidally in time. (D) This sinusoidal twisting field causes both a rotation and a pivoting displacement of the bead. As the bead moves, the cell develops internal stresses which in turn resist bead motions. Here the ratio of specific torque to lateral bead displacement is computed and is expressed as cell stiffness in Pa/nm. (Reproduced with permission of *J. Appl. Physiol.*, Vol. 91, p. 988, © 2001 The American Physiological Society and with permission of *Phys. Rev. Lett.*, Vol. 87, p. 148102-1, © 2001 The American Physical Society.)

specific torque to lateral bead displacements is taken as a measure of cell stiffness (Fabry *et al.*, 2001).

By this technique, it has been previously demonstrated that airway smooth muscle cells in culture exhibit pharmacomechanical coupling to a wide panel of contractile and relaxing agonists (An *et al.*, 2002; Hubmayr *et al.*, 1996). For example, cell stiffness increases in response to agonists reported to increase intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) or inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ) formation and decreases in response to agonists that are known to increase intracellular cAMP or cGMP levels (An *et al.*, 2002; Hubmayr *et al.*, 1996; Shore *et al.*, 1997). Although stiffness is an indirect measure of contractility (Fredberg *et al.*, 1997), changes in cell stiffness range appreciably from maximally relaxed to maximally activated states (Fabry *et al.*, 2001), and such stiffening responses

require, as in intact tissues, actin polymerization as well as myosin activation (An *et al.*, 2002; Mehta and Gunst, 1999). Indeed, active stresses within individual airway smooth muscle cells, as measured by traction microscopy, span a similarly wide range (Figure 1.3) and closely track changes in cell stiffness as measured by MTC (Wang *et al.*, 2002). Altogether, the mechanical responsiveness of airway smooth muscle cells measured in culture is consistent with physiological



**Figure 1.3** Airway smooth muscle cell exerts traction upon an elastic substrate. A representative changes in traction field of a single human airway smooth muscle cell in response to isoproterenol at (A) 0  $\mu\text{M}$ , (B) 0.1  $\mu\text{M}$ , (C) 1  $\mu\text{M}$  and (D) 10  $\mu\text{M}$ . The traction field was computed from the displacement field using Fourier transform traction cytometry (FTTC) (Butler *et al.*, 2002; Tolic-Norrelykke *et al.*, 2002; Wang *et al.*, 2002). The cell boundary is shown by the white line. Colors show the magnitude of the tractions in Pascal (Pa) (see color scale). Arrows show the direction and relative magnitude of the tractions. In general, the greatest tractions are at the cell periphery and directed centripetally. *Inset*. A phase-contrast image of the respective airway smooth muscle cell. Scale bar: 50  $\mu\text{m}$ . (Reproduced with permission of *Am. J. Respir. Cell Mol. Biol.*, Vol. 35, p. 59, © 2006 The American Thoracic Society.) (For a colour reproduction of this figure, please see the colour section, located towards the centre of the book).

responses measured at tissue and organ levels (Fredberg *et al.*, 1996; Mehta and Gunst, 1999). As such, these biophysical methods are unparalleled in their ability to characterize mechanical properties of airway smooth muscle at the level of the single cell *in vitro*.

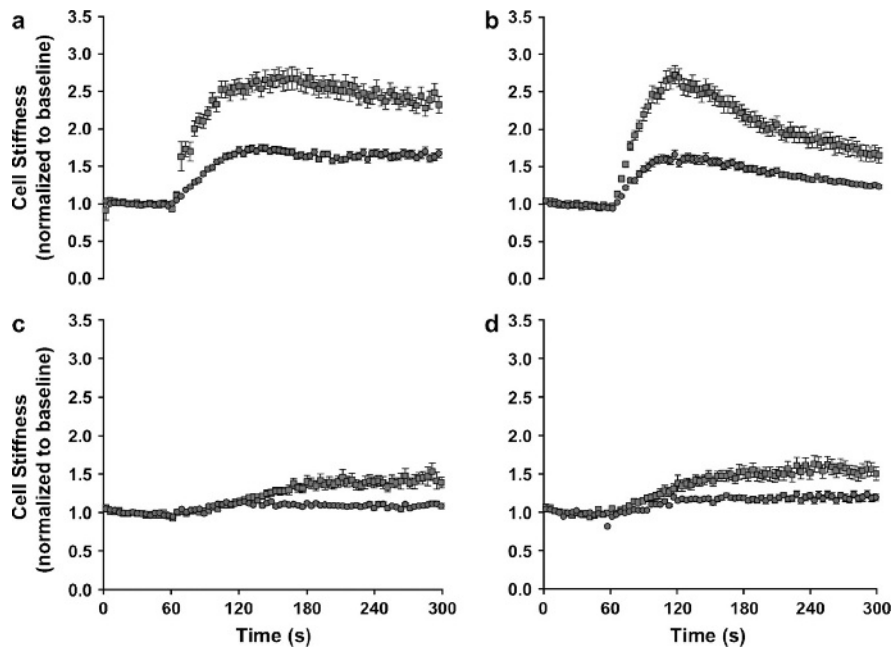
Does mechanical responsiveness of the airway smooth muscle cell predict airway hyperresponsiveness? To address this question, we have recently contrasted the biophysical properties of the airway smooth muscle cell isolated from the relatively hyporesponsive Lewis rat with the relatively hyperresponsive Fisher rat (An *et al.*, 2006). In agreement with biochemical changes that have been previously reported in these cells (Tao *et al.*, 1999; 2003; Tolloczko *et al.*, 1995), compared with cells isolated from Lewis rat, those isolated from the Fisher rat demonstrate in turn greater extent of the stiffening response to a panel of contractile agonists that are known to increase  $[Ca^{2+}]_i$  or  $IP_3$  formation: Fisher airway smooth muscle cells stiffen fast and also stiffen more (Figure 1.4). Furthermore, consistent with these changes in cell stiffness, the relatively hyperresponsive Fisher airway smooth muscle cells also exert bigger contractile forces and exhibit greater scope of these forces (An *et al.*, 2006). Taken together, these findings firmly establish that comprehensive biophysical characterization of bronchospasm in culture is a reality, and these characterizations at the level of the single cell show mechanical responses that are consistent with phenotypic differences in airway responsiveness measured at tissue and organ levels (Dandurand *et al.*, 1993a; 1993b; Eidelman *et al.*, 1991; Jia *et al.*, 1995; Tao *et al.*, 1999).

Like human asthmatics (Johnson *et al.*, 2001; Woodruff *et al.*, 2004), Fisher rats have abundant smooth muscle cells in their airways (Eidelman *et al.*, 1991), and these cells show great capacity to proliferate in culture (Zacour and Martin, 1996). Although these features, together with increased muscle dynamics (shortening velocity as well as contractile force), may account for the enhanced airway responsiveness of Fisher rats, the precise role of airway smooth muscle in the pathogenesis of airway hyperresponsiveness in asthma is ill-defined. It remains equally unclear, although Fisher rats present an attractive model, to what extent this animal model recapitulates the pathophysiology associated with human asthma.

## 1.6 Mechanical plasticity: a nonclassical feature of airway smooth muscle

When activated muscle in the muscle bath is subjected to progressively increasing load fluctuations approaching the magnitude and frequency expected during normal breathing, the muscle lengthens appreciably in response (Fredberg

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**Figure 1.4** Fisher airway smooth muscle cells stiffen fast and also stiffen more. Airway smooth muscle cells isolated from the relatively hypo-responsive Lewis rat (blue closed circles) and the relatively hyper-responsive Fisher rat (red closed squares) were maximally stimulated with a panel of contractile agonists: (A) 5-HT ( $1 \mu\text{M}$ ), (B) bradykinin ( $1 \mu\text{M}$ ), (C) acetylcholine ( $1 \mu\text{M}$ ) and (D) carbachol ( $100 \mu\text{M}$ ). For each agonist, changes in cell stiffness were normalized to the baseline stiffness of each individual cell before stimulation. (Reproduced with permission of Am. J. Respir. Cell Mol. Biol., Vol. 35, p. 57, © 2006 The American Thoracic Society.)

*et al.*, 1999). But when load fluctuations are progressively reduced, the muscle reshortens somewhat but fails to return to its original length. This incomplete to reshortening is not accounted for by muscle injury; the original operating length can be recovered simply by removing the contractile agonist and allowing the muscle a short interval before contracting again. Nor can incomplete reshortening be accounted for by myosin dynamics; myosin dynamics alone predicts complete reshortening when the load fluctuations are removed (Fredberg *et al.*, 1999). Thus, the failure of activated muscle to reshorten completely is evidence of the plasticity of the contractile response. During a sustained contraction, the operational length of the muscle for a given loading, or the force at a given length, can be reset by loading and the history of that loading (Ford *et al.*, 1994; Fredberg

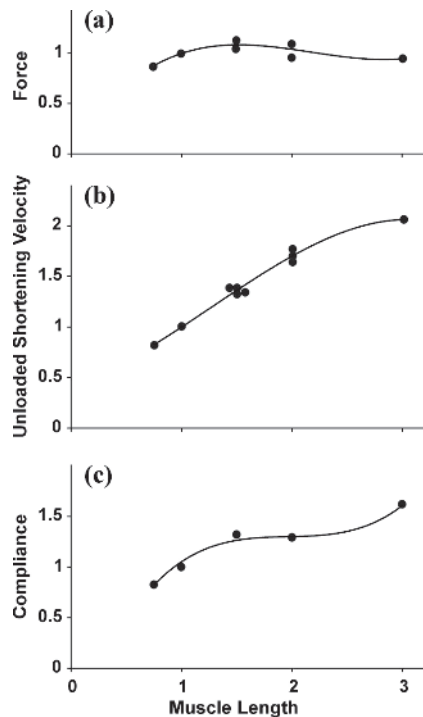
*et al.*, 1997; 1999; Gunst and Wu, 2001; Gunst *et al.*, 1993; Pratusевич *et al.*, 1995; Wang *et al.*, 2001). In healthy individuals, this plasticity seems to work in a favorable direction, allowing activated muscle to be reset to a longer length. The asthmatic, it has been argued, never manages to melt the contractile domain in the airway smooth muscle; therefore, the benefits of this plastic response are not attained.

It is now firmly established that airway smooth muscle can adapt its contractile machinery, as well as the cytoskeletal scaffolding on which that machinery operates, in such a way that the muscle can maintain the same high force over an extraordinary range of muscle length (An *et al.*, 2007; Ford *et al.*, 1994; Fredberg, 1998; Gunst and Wu, 2001; Gunst *et al.*, 1993; 1995; Kuo *et al.*, 2001; 2003; Naghshin *et al.*, 2003; Pratusевич *et al.*, 1995; Qi *et al.*, 2002; Seow and Fredberg, 2001; Seow *et al.*, 2000; Wang *et al.*, 2001); airway smooth muscle is characterized by its ability to disassemble its contractile apparatus when an appropriate stimulus is given, and its ability to reassemble that apparatus when accommodated at a fixed length. When exposed to contractile agonists, airway smooth muscle cells in culture reorganize cytoskeletal polymers, especially actin (Hirshman and Emala, 1999), and become stiffer (An *et al.*, 2002). Although cell stiffening is attributable largely to activation of the contractile machinery, an intact actin lattice has been shown to be necessary, but not sufficient, to account for the stiffening response (An *et al.*, 2002).

The malleability of the cell and its mechanical consequences have been called by various authors mechanical plasticity, remodelling, accommodation or adaptation. Even though the force-generating capacity varies little with length in the fully adapted muscle, the unloaded shortening velocity and the muscle compliance vary with muscle length in such a way as to suggest that the muscle cell adapts by adding or subtracting contractile units that are mechanically in series (Figure 1.5). The mechanisms by which these changes come about and the factors that control the rate of plastic adaptation are unknown, however.

Several hypotheses have been advanced to explain smooth muscle plasticity. Ford and colleagues have suggested that the architecture of the myosin fibres themselves may change (Ford *et al.*, 1994; Kuo *et al.*, 2001; 2003; Pratusевич *et al.*, 1995; Seow *et al.*, 2000), while Gunst and colleagues (Gunst and Wu, 2001; Gunst *et al.*, 1993; 1995) have argued that it is the connection of the actin filament to the focal adhesion plaque at the cell boundary that is influenced by loading history. An alternative notion is that secondary but important molecules stabilize the CSK, and as the contractile domain melts under the influence of imposed load fluctuations, those loads must be borne increasingly by the scaffolding itself, thus reflecting the malleability of the cytoskeletal domain (Fredberg, 2000a; Gunst

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**Figure 1.5** Mechanical plasticity of the airway smooth muscle. (A) Isometric force ( $F$ ), (B) unloaded shortening velocity ( $V$ ), and (C) compliance ( $C$ ) of canine tracheal smooth muscle activated over a range of muscle lengths. Filled circles represent data modified from Pratusевич et al. (1995) and Kuo et al. (2003), as compiled by Lambert et al. (2004); solid lines are third-order polynomial functions adjusted to the original data (Silveira and Fredberg, 2005). (Reproduced with permission of Can. J. Physiol. Pharmacol., Vol. 83, p. 924, © 2005 NRC Canada.)

*et al.*, 1995; Halayko and Solway, 2001; Wang and Bitar, 1998). In that connection, a role for the Rho-A pathway has been suggested (Halayko and Solway, 2001; Mehta *et al.*, 2000), and some evidence now suggests that the p38 MAP kinase pathway may be involved (Lakser *et al.*, 2002). For example, airway smooth muscle incubated with an inhibitor of the p38 MAP kinase pathway demonstrates a greater degree of fluctuation-driven muscle lengthening than does control muscle, and upon removal of the force fluctuations it remains at a greater length. Moreover, force fluctuations themselves activate the p38 MAP kinase pathway. It is noteworthy in that connection that heat-shock protein 27 (HSP27), a downstream target of Rho and p38, has been implicated as an essential element in cytoskeletal remodelling of the airway smooth muscle cell (An *et al.*, 2004; Gerthoffer and

Pohl, 1994; Hedges *et al.*, 1998; 1999; 2000; Yamboliev *et al.*, 2000). These findings are consistent with the hypothesis that stress-response pathways may stabilize the airway smooth muscle CSK and limit the bronchodilating effects of deep inspirations.

## 1.7 Recent observations

Recently, we have made a series of observations in a number of different cell types and reported a functional assay that probes the discrete molecular level remodelling dynamics of the CSK (An *et al.*, 2004; 2005; Bursac *et al.*, 2005; 2007). This assay is based on *spontaneous* nano-scale movements of an individual RGD-coated microbead tightly anchored to the CSK (Figure 1.6): we reasoned that the bead can move spontaneously only if the microstructure to which it is attached rearranges (remodels), and we quantified these motions by calculating its mean square displacement ( $\text{MSD}_b$ ),

$$\text{MSD}_b(\Delta t) = \langle (r(t + \Delta t) - r(t))^2 \rangle \quad (1)$$

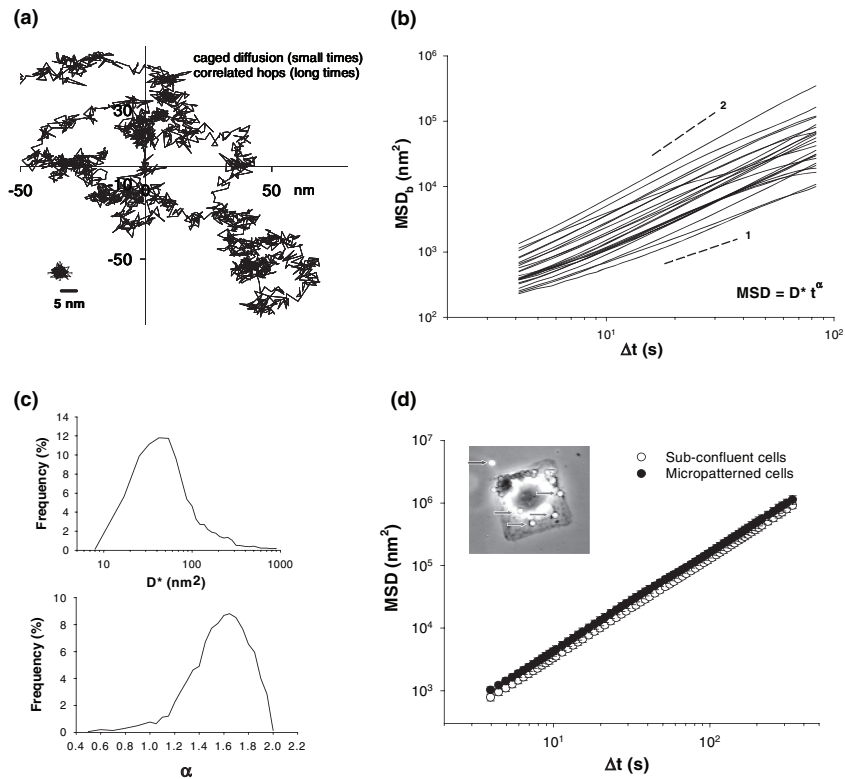
where  $r(t)$  is the bead position at time  $t$ ,  $\Delta t$  is the time lag ( $\Delta t = 1/12\text{s}$ ), and the brackets indicate an average over many starting times (Bursac *et al.*, 2005; 2007). The limit of resolution in our system is on the order of  $\sim 10$  nm, but for  $\Delta t \sim 4$  s most beads had displaced a much greater distance. Accordingly, we analysed data for time lags greater than 4 s and up to  $t_{\text{max}}$ . As shown below, MSD of most beads increases with time according to a power-law relationship.

$$\text{MSD}(\Delta t) = D^*(\Delta t / \Delta t_0)^\alpha \quad (2)$$

The coefficient  $D^*$  and the exponent  $\alpha$  of an individual bead are estimated from a least-square fit of a power-law to the MSD data for  $\Delta t$  between 4 s and  $t_{\text{max}}/4$ . Here we take  $\Delta t_0$  to be 1 s and express  $D^*$  in units of  $\text{nm}^2$ . As shown in Figure 1.6, the ensemble average of all  $\text{MSD}_b$  (MSD) increased faster than linearly with time ( $\sim t^{1.6}$ ), exhibiting superdiffusive motions. Such anomalous motions were also observed on cells seeded on a micropatterned substrate on which a cell could adhere but not crawl (Bursac *et al.*, 2007; Parker *et al.*, 2002). Taken together, unlike simple, diffusive, thermal Brownian motion that increases its MSD linearly with time (Kubo, 1986), *spontaneous* motions of an individual RGD-coated bead are nonthermal in nature and, instead, consistent with the notion that these anomalous motions report molecular-level reorganization (remodelling) of the underlying CSK (An *et al.*, 2004; 2005; Bursac *et al.*, 2005; 2007).

## 1.7 RECENT OBSERVATIONS

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**Figure 1.6** Cytoskeleton remodeling of the airway smooth muscle cell. (A) Spontaneous motions of a representative bead show intermittent dynamics, with periods of confinement alternating with hopping; a bead glued to the coverslip is taken to represent the upper limit of measurement noise (bottom left). (B)  $MSD_b$  calculated from Equation 1 is shown for representative beads. (C) The histograms of diffusion coefficient  $D^*$  and exponent  $\alpha$  estimated from a least-square fits of a power-law (Equation 2) to the  $MSD_b$  data. (D) Ensemble average of all  $MSD_b$  ( $MSD$ ) increased faster than linearly with time ( $\sim t^{1.6}$ ); beads attached to a cell seeded on a micropatterned substrate ( $50 \mu\text{m} \times 50 \mu\text{m}$ ), on which it could adhere but not crawl, exhibited the same anomalous motions. (Reproduced with permission of *Biochem. Biophys. Res. Comm.*, Vol. 355, p. 326, © 2007 Elsevier Inc., and with permission of *Nature Mater.*, Vol. 4, p. 559, © 2005 Nature Publishing Group.)

By this method, we have demonstrated that the rate of cytoskeletal remodelling is appreciably different between airway smooth muscle cells isolated from the relatively hypo-responsive Lewis rat and those from the relatively hyper-responsive Fisher rat: Fisher cells exhibit faster remodelling dynamics (An *et al.*, 2006). Furthermore, such remodelling is dependent on the levels of intracellular ATP

content (An *et al.*, 2006; Bursac *et al.*, 2005) and also becomes progressively slow with phosphorylation of HSP27 (An *et al.*, 2004). Indeed, evidence supporting the notion of a highly malleable cell is accumulating rapidly, but a molecular basis to explain this malleability is only beginning to emerge. Most recently, we observed that, in response to a transient stretch-unstretch maneuver with zero residual macroscale strain, the airway smooth muscle cell promptly fluidizes and then slowly re-solidifies (Trepap *et al.*, 2007). At the same time, the rate of spontaneous nano-scale structural rearrangements promptly accelerates and then slowly decays in a scale-free manner (Trepap *et al.*, 2007). Taken together, these findings suggest that fluidization provides freedom of the cell to reorganize contractile units, stress fibers and focal adhesions in response to mechanical stress (Trepap *et al.*, 2007). Regardless of the specific molecules and mechanisms invoked to explain the plasticity of the contractile responses, therefore, the melting of the contractile domain would appear to be a necessary (or permissive) event, but one that by itself is not sufficient to explain the effects of the history of tidal loading. How these molecular changes and malleability of the airway smooth muscle cell, in turn, correlate with the progression of asthma pathophysiology are currently under investigation in our laboratories.

## 1.8 Future directions

To understand the multifaceted problem of airway hyperresponsiveness in asthma, an integrative understanding that brings together a diversity of factors is essential. We have outlined here an emerging picture of smooth muscle biophysics as it relates to excessive airway narrowing in asthma, but we need to keep in mind that asthma is a chronic inflammatory disorder; therefore, understanding the impact of inflammatory remodelling of the airway wall and the airway smooth muscle cell on disease presentation is vital. Fortunately, with recent technological advances, we are now equipped with both biochemical and biophysical tools to address nagging questions that have often separated the fields of airway biology and smooth muscle biophysics.

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