

Airway Hyperresponsiveness: From Molecules to Bedside Invited Review: The first three minutes: smooth muscle contraction, cytoskeletal events, and soft glasses

Susan J. Gunst¹ and Jeffrey J. Fredberg²

¹Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana 46202; and ²Harvard School of Public Health, Boston, Massachusetts 02067

Gunst, Susan J., and Jeffrey J. Fredberg. Invited Review: The first three minutes: smooth muscle contraction, cytoskeletal events, and soft glasses. *J Appl Physiol* 95: 413–425, 2003; 10.1152/jappphysiol.00277.2003.—Smooth muscle exhibits biophysical characteristics and physiological behaviors that are not readily explained by present paradigms of cytoskeletal and cross-bridge mechanics. There is increasing evidence that contractile activation of the smooth muscle cell involves an array of cytoskeletal processes that extend beyond cross-bridge cycling and the sliding of thick and thin filaments. We review here the evidence suggesting that the biophysical and mechanical properties of the smooth muscle cell reflect the integrated interactions of an array of highly dynamic cytoskeletal processes that both react to and transform the dynamics of cross-bridge interactions over the course of the contraction cycle. The activation of the smooth muscle cell is proposed to trigger dynamic remodeling of the actin filament lattice within cellular microdomains in response to local mechanical and pharmacological events, enabling the cell to adapt to its external environment. As the contraction progresses, the cytoskeletal lattice stabilizes, solidifies, and forms a rigid structure well suited for transmission of tension generated by the interaction of myosin and actin. The integrated molecular transitions that occur within the contractile cycle are interpreted in the context of microscale agitation mechanisms and resulting remodeling events within the intracellular microenvironment. Such an interpretation suggests that the cytoskeleton may behave as a glassy substance whose mechanical function is governed by an effective temperature.

mechanical plasticity; latch state; actin cytoskeleton; mechanotransduction; glass hypothesis

TO FASHION A WORK OF GLASS, a glassblower must heat the object, shape it, and then cool it down. A new perspective on cytoskeletal biology now suggests that the smooth muscle cell may modulate its mechanical properties and remodel its internal structures in much the same way. However, instead of changing thermodynamic temperature, the smooth muscle cell is proposed to modulate an effective temperature, called the “noise” temperature, representing the level of molecular jostling (equivalently, microscale noise or agitation) present in the intracellular microenvironment. Clues in the literature that are now being pieced together hint at the following picture (18, 20). The relaxed unactivated smooth muscle cell is found to be in a state that, by criteria described below, is seen as being relatively “cold,” but with the onset of contractile stimu-

lation the cell very rapidly becomes “hot” (Fig. 1). After this hot initial transient, the cell then begins to “cool” if the contractile stimulation is sustained, becoming gradually colder and colder until, eventually, it approaches a steady state that for all practical purposes approximates a “frozen” state not only mechanically but also, to an appreciable extent, biochemically and metabolically. Moreover, recent data suggest that this frozen state preserves an indelible memory of events transpiring in the early history of the contraction (Fig. 2) (23, 33, 64, 90).

These statements might be thought of in purely metaphorical terms of course, and every metaphor if pushed far enough will fail. In this case, however, they are not at all metaphorical. Rather, they comprise a precise statement of the glass hypothesis, which, in turn, makes predictions that are mechanistic, quantitative, and testable. This is possible because the noise temperature, as described below, turns out to be both a measurable quantity (18) and one that

Address for reprint requests and other correspondence: J. J. Fredberg, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115 (E-mail: jfredber@HSPH.harvard.edu).

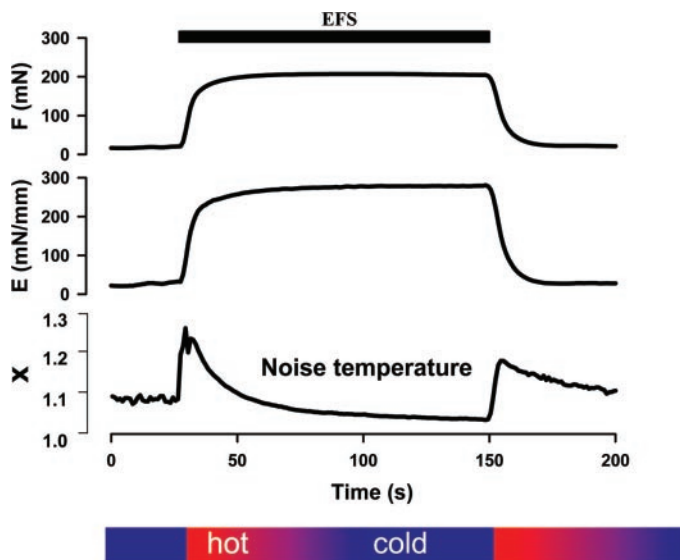


Fig. 1. The evolution of tension (F), stiffness (E), and noise temperature (X) in a representative canine tracheal smooth muscle strip activated by electric field stimulation (EFS, black bar). Tension and stiffness increase throughout the contractile event but do so most rapidly during the early hot epic. At that time, the unloaded shortening velocity (Fig. 5), the rate of ATP utilization (Fig. 4), and the cross-bridge cycling rates peak and then progressively diminish. Adapted from Ref. 25.

varies in a systematic fashion during the contractile event (Fig. 1).

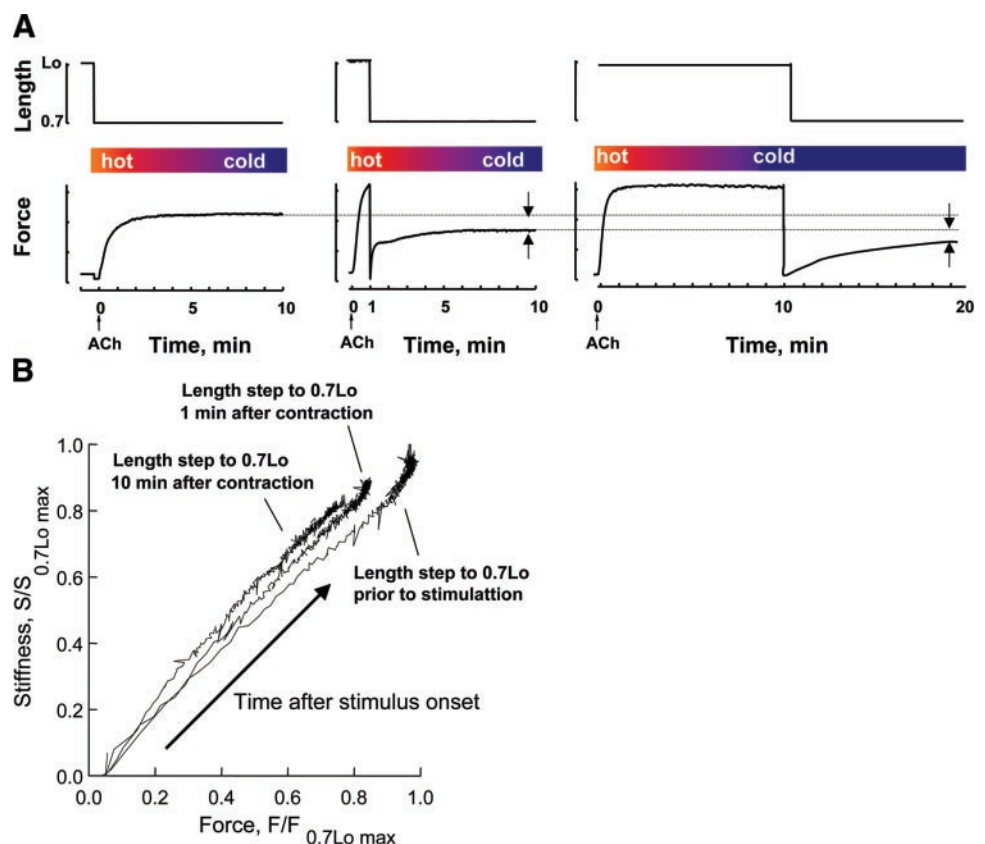
We take the glass hypothesis seriously not because it is well established, which it is not, but rather because

it is interesting. The hypothesis becomes interesting because it presumes to explain in one stroke the abilities of the cytoskeleton to deform, to flow, and to remodel, and in those regards it has already scored some successes and provided a coherent conceptual framework (18, 20). These mechanical processes, in turn, are fundamental to a variety of higher integrative cell functions, including cell contraction, adhesion, spreading, crawling, invasion, wound healing, and division, and have been implicated as well in mechano-transduction, regulation of protein and DNA synthesis, and programmed cell death (42). It becomes interesting, therefore, to follow the consequences of the hypothesis and see where they may lead.

The gap of understanding. Although evidence available to date suggests that the glass hypothesis might be applicable to a variety of cell types (18), we restrict attention here to the airway smooth muscle cell and the first 3 min of the contractile event; in format, of course, we have borrowed freely from Steven Weinberg and his book of the same name (97). Accordingly, our objective in this review is to trace the evolution of molecular events that are thought to transpire during smooth muscle activation and, at the same time, to view these events through the lens of the glass hypothesis. We juxtapose these contrasting perspectives for two reasons.

First, it has become increasingly clear in recent years that contractile activation of the smooth muscle cell comprises events that reach far beyond the immediate issues of thick filament (myosin) regulation, thin filament (actin) regulation, and the resulting actomy-

Fig. 2. Airway smooth muscle preserves an indelible memory of events transpiring in the early history of the contraction. **A**: length decrease imposed 1 min after muscle activation with acetylcholine (ACh), just as the hot epoch is ending, depresses the final active tension relative to a length step imposed just before muscle activation. When the length step is imposed later in the contraction (10 min), the tension depression is greater. **B**: relationships between muscle stiffness and force during the tension development following the length steps illustrated in **A**. In each case, muscle stiffness increases relative to tension during the plateau phase of contraction. Stiffness during tension redevelopment is greater when the length step is performed 10 min after stimulation than 1 min after stimulation, consistent with increasing cytoskeletal rigidity and reduced plasticity as the contraction progresses. The glass hypothesis proposes that the hot epoch is permissive of rapid adaptation of the cytoskeleton to its current operating length, with these early events subsequently becoming frozen during the cold epoch that follows. L_o , optimal length. Adapted from Ref. 33.



osin interaction. To be sure, the actomyosin interaction is the central player in the contractile event, but the actomyosin interaction and its regulatory molecules have been studied extensively and are now known to represent only a handful out of scores of accessory, scaffolding, and focal adhesion plaque molecules whose polymerization, activation, and orchestrated mechanical actions, taken together, comprise the contractile event (11, 27, 58, 61, 78). Our understanding of the laws that govern the integrated actions of these numerous individual processes remains quite fragmentary, however. The manner in which the multitudes of contractile, cytoskeletal, and associated proteins interact with one another within the dynamic microenvironment of the cell body so as to produce integrated mechanical effects (e.g., cellular contraction, stiffness, or remodeling) remains a major open question (21). As described below, the glass hypothesis and the noise temperature appear to provide at least the hope of a firm, integrative framework within which those processes might fit. In addition, the noise temperature is of special interest due to its relationship to both the mechanical and molecular changes that are involved and the sensitivity and speed of the experimental probes that are now available (18, 19).

The second reason to juxtapose these perspectives is simply the converse of the first. If the glass hypothesis is interesting because it might set physical limits on mechanical and underlying molecular processes and because it promises to place these processes into a firm integrative framework, it is important to recognize that the hypothesis itself is quite limited. As presently formulated, it does not point to any specific molecular interaction but rather deals only with generic features of such interactions (16, 18, 20, 73, 74, 88, 98).

This leaves a wide explanatory gap. And perhaps it is only when molecular and integrative perspectives are taken together that we can suddenly appreciate just how far we are from understanding the contractile event. In that regard, it is a bit like viewing an impressionistic painting: too close and all one sees is blotches. But farther away, at the right level, the image snaps into focus. In the case at hand, here what snaps into focus, at least in part, are plausible insights into longstanding questions or nagging unexplained observations and, more importantly, new questions never before posed. In addition to putting the functional roles of specific molecules into an integrative context, as a practical matter there is also reason to believe that the glass hypothesis might provide insights into pathological mechanisms, including airway hyperresponsiveness in asthma, the inability of asthmatic patients to dilate their airways with a deep inspiration, and the plasticity and remodeling of the airway smooth muscle cell (23, 25, 33, 52, 59, 64, 67, 90).

Important ideas that came before. Ideas already well established in the literature go a long way toward explaining the biophysics of the cytoskeletal system. To explain cytoskeletal stiffness, in particular, the gel paradigm (62, 77, 78) emphasizes graded degrees of gelation (vs. solation) associated with varying degrees

of polymerization and cross-linking; these processes are sometimes described in terms of the percolation phenomenon (12, 22). The tensegrity paradigm of Ingber (41), by contrast, has had a stormy history but very recently has garnered substantial experimental support (75, 76, 92, 93); tensegrity emphasizes the contribution of a tensed filamentous lattice. Taken together, the mechanisms embodied by these paradigms are complementary, fundamental, and important, but they are also in their entirety static; they address only the matrix stiffness, which is a static mechanical property, and they imagine the cytoskeleton to be at every instant at a thermodynamic equilibrium or to be passing slowly through a sequence of equilibrium states. By their very nature, therefore, neither can speak to the issue of cytoskeletal dynamics, which is to say rates, rate processes, and departures from thermodynamic equilibrium. Accordingly, factors falling outside their scope necessarily include dynamics of protein-protein interactions, velocities (be they rate of matrix deformation, cell shortening velocity, crawling or transport velocities, and the relationship of each velocity to a relevant force), turnover and remodeling of cytoskeletal constituents, matrix disorder, and dynamic equilibria that exist far away from thermodynamic equilibrium. Although each of these factors could be invoked on an ad hoc basis, and have been, to account for changes between what have been taken to be static equilibrium states, dynamics is not an intrinsic part of either of these paradigms and, in principle, falls quite beyond their reach. Although far from being understood, all of these dynamic factors fall naturally within the context of the cytoskeleton as a glassy system.

WHAT'S SO SPECIAL ABOUT SOFT GLASSY MATERIALS?

Glasses are substances with the structural disorder of a liquid but the stiffness of a solid. The physics of glasses in general, and of soft glasses in particular, are much studied but remain poorly understood (88). The class of soft glassy materials comprises a collection of substances that, on first glance, is astounding in its diversity; the group includes foams (e.g., shaving cream), pastes (e.g., toothpaste), colloids, emulsions, slurries, and, importantly, a variety of living cells, including the human airway smooth muscle cell (18, 73, 74, 88, 98). Yet each is a soft glass, and, as described below, their mechanical behaviors are very much alike.

Sollich (73) reasoned that, because the materials comprising the class are so diverse, their common mechanical behavior must be not so much a reflection of specific molecules or molecular mechanisms, for these differ from system to system. Instead, he proposed that their common mechanical behavior might be a reflection of generic system properties that play out at some higher level of structural organization (73). The generic features that these materials share are that each is composed of elements that are discrete, numerous, and aggregated with one another via weak interac-

tions. In addition, these materials exist far away from thermodynamic equilibrium and are arrayed in a microstructural geometry that, at some level or other, expresses metastable arrangements and is inherently disordered.

Messy mechanical systems. Disorder in this context is not at all meant to imply utter chaos or a complete absence of systematic structure, as in a fluid. Rather, like other soft glasses, the smooth muscle cell is a somewhat disordered mechanical system, although the degree of disorder is variable. As distinct from striated muscle, for example, which is highly ordered, there is abundant evidence in the literature demonstrating that the cytoskeletal matrix of smooth muscle is quite disordered; it is, after all, its amorphous structure that gives "smooth" muscle its name. Thus a primary conceptual implication of the glass hypothesis, and one that represents a major point of departure from contemporary mainstream biological thinking, is that the extent of disorder of the cytoskeletal matrix, as well as metastability of its structural elements, are central features whose modulation might play central roles in cytoskeletal mechanics.

Along with those generic features are the empirical criteria that define this class. Primary among these are that these materials are all very soft (in the range of Pa to kPa, or softer than a very soft rubber by more than three orders of magnitude) and that the stiffness of the matrix increases with frequency (f) of an imposed deformation in a very special way, namely, a weak power law f^{x-1} ; in the theory of soft glasses, described below, x turns out to be the noise temperature and, as such, can be determined by measuring this exponent. Finally, in these materials, the internal frictional stress is proportional to the elastic stress but, roughly speaking, is typically one-tenth as large (73, 74). Data in the literature thus far establish that six mammalian cell types, including the airway smooth muscle cell, satisfy all of the mechanical criteria as well as all of the generic features that define the class of soft glassy materials. Accordingly, our working hypothesis is that the cytoskeleton of the living cell is indeed a soft glassy material.

By contrast, ordinary polymers as well as striated muscles do not behave in these ways at all. For example, for an ordinary polymer, the graph of stiffness vs. the frequency at which the measurement is made approximates a staircase function (although with well-rounded shoulders), with one or a few plateaus being evident (95). The frequency marking the transition between adjacent plateaus is the shadow of an underlying molecular relaxation time scale and identifies a particular molecular rate process. In tissues, for example, transitions are evident and associated relaxation times are linked to cross-bridge cycling rates (45, 46, 51). For cells in culture, by contrast, as for other soft glasses, no such features are evident (18). Instead, when cytoskeletal matrix stiffness is measured over a very wide range of frequencies, stiffness increases with frequency in a smooth featureless fashion and no special relaxation time stands out. By mechanisms that

remain obscure, within the cell relaxation, processes are present simultaneously at all time scales. As such, no one molecular mechanism, or even several distinct molecular mechanisms, can explain the mechanical behavior of these systems (73). A multitude of distinct molecular mechanisms cannot be ruled out, but such an interpretation is unsatisfying because it raises more questions than it answers. An alternative hypothesis, and the one that is addressed here, is that there is something else, a common factor at perhaps a different level altogether, that might unify these observations.

Where the trail leads. The behavior of glasses in general, and soft glassy matter in particular, remains one of the outstanding unsolved problems in the field of condensed matter physics and thus places the glass hypothesis of cytoskeletal biophysics at an intersection of open questions in disciplines previously thought of as being distinct. Although most biologists may have a hard time interpreting the significance of the particular biophysical properties that are emphasized above, as opposed to the more familiar physiological properties of muscle, the experimental facts are that this is where the data trail seems to lead; later in this review, we try to link some of these novel properties to the more traditional ideas.

Accordingly, for soft glasses in general and for cells in particular, the interesting questions become these. How can these substances be so very soft and yet retain the properties of a solid? Each of these materials is in a continuous state of remodeling, but how do they manage to remodel so readily, and what is the remodeling mechanism? Is the rate of remodeling governed by an effective temperature, as described below? Although it is not at all accepted to be important in the case of cells, in all of the other materials belonging to the class it seems that metastability of the interactions and disorder of the matrix are central factors; however, why might this be so, and do these factors actually play a role in the cell? Why is the frictional stress in the matrix frequency insensitive, why does it covary with the elastic stress, and why is it not governed by an independent viscous mechanism? And finally, why does stiffness increase with frequency of an imposed deformation as a weak power law?

Measurement. Before moving on, a few words about measurement of the noise temperature are in order. There are several approaches to the measurement of the noise temperature. One approach, and a straightforward one, is to impose small cyclic deformations on the muscle cell and then measure the dependence of cell stiffness on the frequency. This can be done by using magnetic bead twisting or atomic force microscopy in individual adherent cells (1, 18) or cell stretching in isolated cells. A power law results, and the exponent of that power law is simply $x - 1$, thus determining the noise temperature x . Alternatively, at a fixed frequency, one can make a graph of force vs. displacement (in the case of atomic force microscopy or cell stretching) or bead torque vs. bead displacement (in the case of magnetic bead twisting). In either case, an elliptical loop results, and that loop defines a phase

angle that is found to be insensitive to the frequency of the probe. By a simple relationship, that phase angle defines the noise temperature (18). This latter method is useful in tracking changes of the noise temperature with time (Fig. 1). A noise temperature of unity implies a phase angle of zero and a stiffness that is independent of frequency. With increasing noise temperature, the frequency dependence of the stiffness and the phase angle increase. Finally, we point out that the noise temperature x is linked to the hysteresivity (or loss tangent) η by the simple relationship $\eta = \tan \pi(x - 1)/2$ and, as described below, might offer an explanation of its mechanistic basis.

HOPPING EVENTS AND THE NOISE TEMPERATURE

To describe the interaction between the elements within a glassy matrix, the theory of soft glasses proposes that each individual element exists within an energy landscape containing many wells, or traps, each of differing depth E (Fig. 3C). These traps are formed by interactions of the element with neighboring elements (Fig. 3A). In the case of living cells, those traps might be plausibly thought to be formed by binding energies between neighboring cytoskeletal elements, including but not limited to cross-links between actin filaments, cross-bridges between actin and myosin, interactions between other structural proteins, hydrophobic-hydrophilic interactions, charge effects, or simple steric constraints.

An element can escape its energy well and fall into another nearby well; in ordinary window glass, such hopping events are thought to be activated by thermally driven random fluctuations (Fig. 3B). As distinct from ordinary window glass, in a soft glass, each energy well

is imagined as being so deep that the elements are unlikely to escape the well by thermal fluctuations alone. Instead, elements are imagined to be agitated, or jostled, by their mutual interactions with neighboring elements (73). That is to say, random agitation (noise) can excite a metastable element, causing it to hop out of its well, in turn triggering secondary rearrangements and hopping events that ripple through the system (98). A clear notion of the source of the nonthermal agitation remains to be identified, a point to which we return momentarily. However, whatever its particular source might be, the theory holds that this agitation can be represented by an effective temperature or noise level, x . When x is greater than 1, there is sufficient agitation in the matrix that the element can hop randomly between wells and, as a result, the system as a whole can flow and become disordered. In addition, when a hop does occur, whatever elastic strain energy might have been stored in the configuration of the cage microdomain would then be lost, thereby accounting for the tight coupling of mechanical friction to elasticity that is characteristic of these systems. When x approaches 1, however, the elements become trapped in deeper and deeper wells from which they are unable to escape; hopping events virtually cease, and the system exhibits what is called a "glass transition." In this limit, the system approximates an ideal elastic solid, i.e., one that is described by Hookean elasticity, expresses no internal friction, and undergoes no remodeling. That is to say, when the noise temperature approaches unity, the system approaches a frozen state.

As such, when the noise temperature is greater than unity, the following picture is imagined. Although the

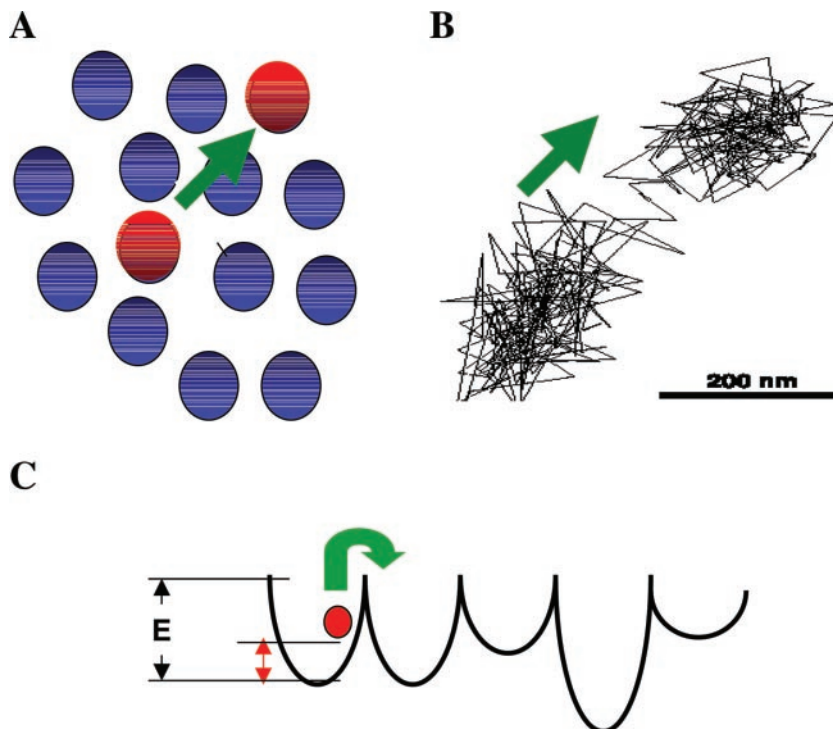


Fig. 3. Caged diffusion and hopping. *A*: each structural element is imagined as being trapped in a cage formed by neighboring elements. However, with sufficient agitation, the element can hop (green arrow) from one cage to another. Adapted from Ref. 20. *B*: caged diffusion and a hopping event demonstrated experimentally in a colloidal glass (96). *C*: each cage can be represented as an energy well of depth E , with hops corresponding to transitions from one well (cage) to another. The rate of hopping is proposed to increase with increasing noise temperature (73, 74). When a hop does occur, any elastic strain energy that is stored (red arrow) is dissipated.

state of least free energy would tend toward order (although not absolutely so), the interactions between elements in a glass are too complex and too weak to form ordered structures spontaneously. Rather, as the system is rapidly quenched, each element finds itself trapped in a cage formed by its neighbors, like finding oneself suddenly trapped by adjacent passengers in a crowded subway car (Fig. 3A). In that cage (equivalently, an energy well), elements are trapped away from energy minima and, therefore, the system is not at a thermodynamic equilibrium. Instead, the matrix is in a continuous state of remodeling, continuously exploring different internal microstructural configurations, doing so at a rate set by the noise temperature but never finding an equilibrium configuration.

Noise temperature and its modulation. The effective noise temperature x is a temperature to the extent that the rate at which elements can hop out of a trap assumes the form $\exp(-E/x)$, where x takes the usual position of a thermal energy (kT) in the familiar Boltzmann exponential and E is the well depth. By analogy, x has been interpreted by Sollich et al. (73, 74) as reflecting jostling of elements by an unidentified but nonthermal origin. The absence of a clear mechanistic basis of microscale agitation has been considered a central conceptual weakness of the theory of soft glasses, however (18, 74).

It has been speculated that this central weakness might be easily resolved in the case of living cells, as opposed to the inert materials for which the theory of soft glasses was originally devised, because there is an obvious and ready source of nonthermal energy injection, namely, those proteins that go through cyclic conformational changes and thus agitate the matrix by mechanisms that are ATP dependent (18). But would ATP-dependent agitation of this kind be big enough to make a difference? In an adult at rest, the energy metabolism is ~ 100 W and corresponds to an ATP utilization of 10^{21} molecules/s or roughly 10^7 molecules/s per cubic micrometer of cytoplasm. The energy released by ATP hydrolysis is ~ 25 kT per event, and about one-half of that corresponds to mechanical energy release during the conformational change of the protein; associated molecular deformations are in the range of 1–10 nm (38). Such deformation events might be thought of as agitating neighboring molecules.

How close are these neighbors? In the case of myosin II, if the intracellular concentration were 20 μ M, then we would expect to find on the order of 10^4 myosin II molecules per cubic micrometer of cytoplasm, which translates into a spacing between nearest neighbors of ~ 60 nm on average. Accordingly, molecular spacings of myosins are comparable in size to molecular dimensions and comparable in size to molecular deformations during the conformational change [10 nm (38)]. The cytoplasm is seen therefore as being both a very crowded space (30) and one that is being agitated violently, with local energy release per event being 10 times larger than kT and occurring at a high rate. If each agitated molecule were trapped in an energy well whose depth is set by van der Waals forces, for exam-

ple, then well depth would be on the order of 10 kT or more, and the distribution of depths would be expected to be quite broad due to inherent matrix disorder. To a first approximation, then, these ideas, taken together, are consistent with premises that underlie the theory of soft glasses as described by Sollich, and they suggest an effective matrix temperature in the living cell that might be substantially greater than the thermodynamic temperature. Nonetheless, the relevance of ATP-dependent agitation to the noise temperature remains at this time only a possibility.

MOLECULAR TRANSITIONS IN THE SMOOTH MUSCLE CYTOSKELETAL DURING THE CONTRACTILE EVENT

How might glassy behavior relate to specific molecular events occurring during smooth muscle contraction and relaxation? To put a molecular face on this picture, we will consider molecular events that are known to occur within the cell over a short time period, say the first few minutes after contractile activation, and interpret these events within the framework of a soft glassy system. We note at the outset that the rate at which a contractile event proceeds is variable because it depends innately on the particular muscle in question and the mode of its activation.

There is wide agreement regarding many aspects of molecular transitions that occur within the contractile apparatus during the activation-deactivation cycle of a smooth muscle cell. In contrast, other molecular events that occur within the cytoskeletal system are more controversial, or even hypothetical, given the limited data currently available. For present purposes, we will integrate all such events to question the extent to which they might or might not fall plausibly under the rubric of "glassy" behavior of the smooth muscle cell.

Actomyosin cross-bridge interactions. Many aspects of the kinetic and molecular properties of actomyosin cross-bridge interactions have been extensively investigated and are widely accepted. Intracellular Ca^{2+} , myosin light-chain phosphorylation, myosin ATPase activity, and cross-bridge cycling rates are low in smooth muscle cells at rest (44). Cross bridges are believed to remain primarily detached, although a small proportion of attached cross bridges probably persist and may continue to cycle at a slow rate. Because the number of actomyosin interactions is small, muscle tension and stiffness are small compared with their maximum attainable values, the rate of myosin ATPase activity is low but nonzero, and the noise temperature falls somewhere in the middle of its attainable range and is somewhat larger than unity (Figs. 1 and 4). Note that muscle tension, stiffness, and ATPase activity (Figs. 1, 2, 4, and 5) depend on the number of molecular interactions that are participating, whereas the noise temperature is an index of the rate of turnover of interactions but is independent of the number of such interactions.

Activation of the cell via external stimuli causes a rise in intracellular Ca^{2+} , the activation of myosin light-chain kinase, myosin light-chain phosphoryla-

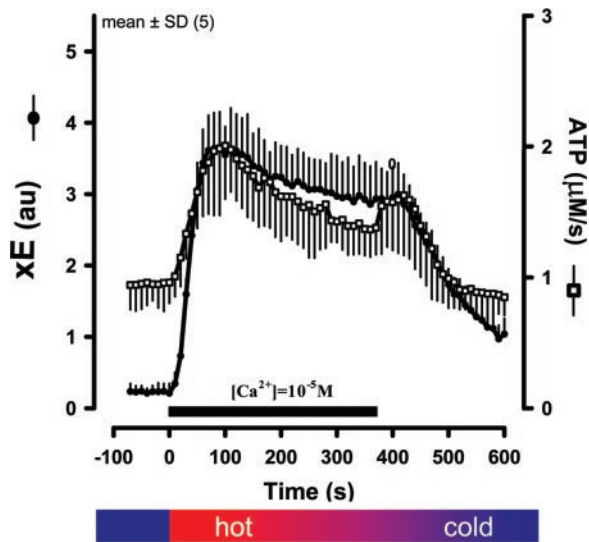


Fig. 4. Activation of a permeabilized (Triton X-100) canine tracheal smooth muscle bundle with the rate of ATPase activity measured by NADH fluorimetry (*right y-axis*). The muscle is activated by increasing the concentration of intracellular free Ca^{2+} (black bar). The product of the x (which is a measure of the intrinsic reaction rate) and the muscle E (which is a measure of the number of interactions) is shown on the *left*. Both xE and ATPase peak simultaneously and then decrease in concert. To compare the respective time course of the mechanical (xE) and metabolic (ATPase activity) responses, data are matched at their peak values; the high-baseline value of ATPase activity measured by NADH fluorimetry is thought to be artifactual. au, Arbitrary units. Adapted from Ref. 25.

tion, the engagement of cross bridges and rapid cross-bridge cycling (Fig. 6). Depending on the muscle load, these events result in rapid tension development or active cell shortening. The attachment of cross bridges that occurs on activation of the cell causes an immediate increase in the stiffness of the cytoskeletal system (33, 39, 43, 49). This increased stiffness persists for the duration of the period of contractile activation and even increases during the tonic phase of contraction in airway smooth muscle (Fig. 2) (33, 34). The rapid increases in stiffness and tension are accompanied by rapid increases in the rate of ATP utilization, the velocity of unloaded shortening, and the noise temperature (Figs. 1, 4, and 5), all of which are suggestive of rapid rates of actomyosin cross-bridge cycling. Although each of these measures varies with different tissues and preparations, they peak very soon after cell activation, and this period corresponds to the “hottest” epoch in the contractile event.

In airway, vascular, and other smooth muscle tissues, this hot epoch is followed by a period of partial deactivation of the contractile apparatus even though the stimulus is maintained: intracellular Ca^{2+} falls, and myosin light-chain phosphorylation, myosin ATPase activity, velocity of unloaded shortening, and the noise temperature all decline (15, 25). The ability of the contractile system to respond to changes in the physical environment of the cell by rapid shortening or tension development also declines, even though the stiffness of the smooth muscle cell and its ability to sustain high levels of tension remain high. These prop-

erties characterize what has been termed the “latch” state because they supposedly represent a state in which cross bridges remain attached but no longer cycle rapidly (55). Murphy (54, 55) has emphasized that the latch state is biologically economical in that it allows the smooth muscle cell to maintain the tone and shape of hollow organs while metabolizing ATP at a rate that is ~ 300 times smaller than the same tension supported by striated muscle. The latch state is now interpreted as being a “cold” contractile state.

Mechanical plasticity of smooth muscle. The actomyosin cross-bridge interaction and associated regulatory processes are necessary to explain tension development and active shortening of the smooth muscle cell, but they are not sufficient to account for all of its contractile properties (32). The plasticity and length-history dependence associated with the contractile response of smooth muscle are well documented (7, 24, 31, 33–36, 50, 64, 90). The response of the smooth muscle cell to any given stimulus depends strongly on its physical conditions before and during the period of contractile activation (Fig. 2). Even in its resting state, the properties of the smooth muscle cell are slowly adjusting and accommodating to changes in the current physical environment (31, 35, 91). Importantly, the state of any system at a thermodynamic equilibrium is set by current conditions only, with the equilibrium distributions of all species, structures, and complexes being determined by the laws of statistical mechanics (97). If the evolution of the contractile event were composed of a sequence of equilibrium states or merely approached an equilibrium state at long times, then events transpiring in the early history of the contraction would necessarily be erased (89, 97). However, in smooth muscle cells, which show substantial mechanical plasticity (Fig. 2), we know this not to be the case. It follows logically, therefore, that departures from thermodynamic equilibrium must play a large role in the evolution of cell mechanical properties throughout the contractile event. More than being just an esoteric point, this idea rules out certain mechanisms of plasticity; gelation, for example, is an equilibrium process and, although it undoubtedly plays a role,

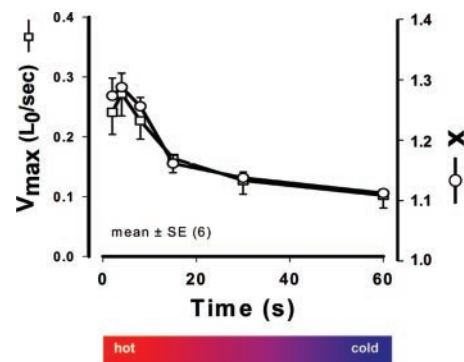


Fig. 5. Unloaded shortening velocity (V_{\max}) and x in maximally activated canine tracheal smooth muscle strips. V_{\max} and x peak simultaneously early in the contractile event and then decrease in concert. Adapted from Ref. 25.

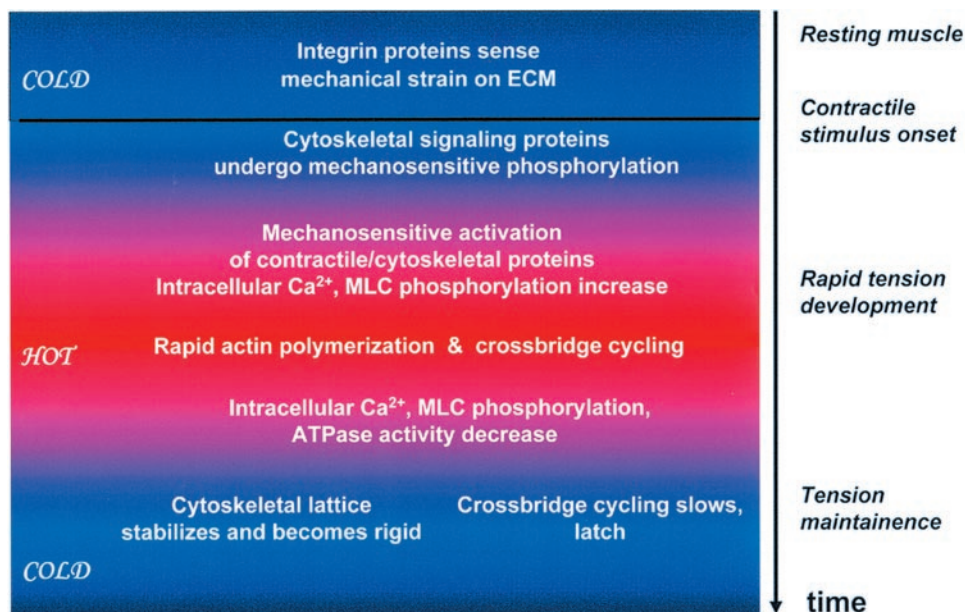


Fig. 6. A summary of major events transpiring during smooth muscle activation. MLC, myosin light chain.

gelation by itself is insufficient to account for mechanical plasticity of the cell.

In that connection, cross-bridge interactions can efficiently and rapidly alter smooth muscle cell length, tension, and stiffness, but by themselves they cannot account for cytoskeletal malleability and its ability to accommodate cell mechanical responses to the physical environment, either while activated or quiescent. Other molecular interactions within the cytoskeleton are likely to be responsible for these properties and consequently to be important determinants of its glass-like properties.

As is documented for other cell types, cytoskeletal architecture of the smooth muscle cell is continuously remodeling and readjusting in response to external physical events as the cell microenvironment changes. Thus, rather than constituting a fixed scaffolding for the sliding of actin and myosin filaments, the structure of the actin filament lattice and its linkages to the external environment of the cell are plastic and mutable, subject to continuous change and adaptation as events from the external world impinge on it (5, 47, 70). However, in contrast to our fairly detailed knowledge of cross-bridge interactions, very little is known about how such cytoskeletal processes might be regulated during the contraction cycle in smooth muscle and the way in which they might interface with the molecular pathways involved in the activation and regulation of cross-bridge cycling. Furthermore, sufficient information is not currently available to link particular mechanical properties of smooth muscle to specific molecular events within the cytoskeleton. However, evidence obtained in other cell types provides a framework for considering how molecular processes within the cytoskeleton could form the basis for the unique mechanical characteristics of smooth muscle.

Molecular basis for the regulation of cell shape and migration. A wealth of data supports a central role for actin filament assembly/disassembly in the generation

of motile force by migrating cells and in regulating cell shape and rigidity (4, 6, 9, 47, 63, 87). Recent discoveries have provided important insights into how cells regulate actin polymerization to control their motility and shape. The Arp2/3 complex, a complex of seven proteins that is expressed ubiquitously in eukaryotic cells, is now recognized as a key regulator of actin polymerization (63). The Arp2/3 complex catalyzes the assembly of actin filament networks at the leading edges of migrating cells by binding to the sides of existing actin filaments and nucleating the formation of new filaments, which provide the motile force for membrane protrusion (53, 81). The cellular localization and activation of the Arp2/3 complex are now attributed largely to the Wiskott-Aldrich syndrome protein (WASP) family of proteins, which includes N-WASP and a related group, the Scars. These proteins bind directly to the Arp2/3 complex and regulate its activation (63). WASP family proteins are recruited to the membrane and activated by a variety of signaling molecules, including phosphatidylinositol 4,5-bisphosphate, the small Rho family GTPase, cdc42, and tyrosine kinases. Thus WASP family proteins provide a mechanism for triggering the initiation of actin polymerization in response to extracellular stimuli.

Mechanical force can be used by the cell as a signal to regulate actin polymerization and the generation of motile force and to strengthen junctions between the cytoskeleton and the extracellular matrix. The application of mechanical force or stretch to integrin receptors stimulates the recruitment of the cytoskeletal linker proteins talin, vinculin, and α -actinin to integrin-extracellular matrix junctions to form and support connections between integrins and actin filaments, resulting in the formation of adhesion complexes capable of exerting and supporting migration force (14, 17, 26, 65, 66). In crawling cells, the Arp2/3 complex also interacts with vinculin at sites of newly engaged integrin proteins, thereby providing a mechanism by which

actin polymerization can be coupled to newly forming adhesion sites (13). Thus the application of either mechanical force or pharmacological stimuli may drive the strengthening of cytoskeletal-membrane junctions involved in the transmission of force, as well as the recruitment of molecules that catalyze actin filament polymerization. The Arp2/3 complex may also interact with actin-filament cross-linking proteins such as filamin to stabilize three-dimensional actin networks and link them to cell membranes (79). Filamin may also participate in the transduction of membrane receptor-mediated signals to the actin cytoskeleton. For example, in fibroblasts subjected to shear stress, the β_1 -integrin subunit associates with filamin directly, signaling the stressed cell to stiffen and render it resistant to subsequent strains (29). Stossel et al. (79) have proposed that this mechanoprotective function of filamin might be especially important in muscle cells.

Cytoskeletal dynamics in smooth muscle. As in migrating cells, the response of the smooth muscle cell to changes in shape imposed by external forces may be mediated by local mechanotransduction events that serve to catalyze actin polymerization and remodeling in regions of the cell subjected to tension or strain. These mechanotransduction events within the cell may enable it to adapt and remodel itself to conform to external forces even when inactive. Signaling events triggered by the pharmacological activation of cells may elicit coordinated responses throughout the cell that fix the conformation of the actin filament lattice and increase its rigidity, as well as fortify the strength of its connections to the exterior of the cell, thus providing a strong and rigid framework for the transmission of force generated by the interaction of myosin motors with actin. Actin polymerization and remodeling may occur preferentially at points of tension or mechanical strain in the cell membrane, and the strengthening of points of tension transmission to the exterior of the cell may occur at discrete points of membrane stress or strain (10).

When the cell is at rest, the rate of cytoskeletal remodeling triggered by mechanotransduction events might be relatively slow, but activation of the cell by pharmacological stimuli may stimulate the rapid remodeling and solidification of the cytoskeletal lattice. The concurrent activation of cross-bridge cycling may also exert mechanical forces on the cell from within that shape the design of the cytoskeletal remodeling process and accelerate the fortification of the actin filament lattice. The rate and extent of cytoskeletal remodeling in any region of the cell may represent an integrated response to local mechanical and chemical events. External forces and internal events might thus combine to determine the rate and direction of localized areas of extension and retraction of the actin cytoskeletal lattice as well as its rigidity, thereby resulting in a smooth muscle cell that can be malleable and adaptable but that can also be rigid and resistant to deformation.

Taken together, evidence available to date is consistent with the idea that the cytoskeleton is most

malleable when the noise temperature is high and much less so when the noise temperature is low (Figs. 1 and 2).

Evidence for cytoskeletal remodeling in smooth muscle during the contractile event. Although there are presently huge gaps in the experimental evidence to support the above model of the molecular basis for the glassy properties of smooth muscle, there is growing evidence that is consistent with it. There is compelling data that the actin cytoskeleton of smooth muscle remains in a dynamic state and that transitions between monomeric (G actin) and filamentous (F) actin are regulated by contractile stimulation. In unstimulated smooth muscle cells, a high proportion (30–40%) of actin is in soluble form, and the activation of smooth muscle cells and tissues stimulates the polymerization of some of this actin into filamentous form (2, 3, 8, 37, 48). In tracheal smooth muscle tissues, ~10–15% of the total cellular actin undergoes polymerization during contractile stimulation (48). Experimental evidence supports a role for actin polymerization in the contractile process that is distinct from the function of actin filaments in interacting with myosin and cross-bridge cycling. The inhibition of actin polymerization by latrunculin, which binds to G-actin monomers and prevents their incorporation into F actin, inhibits tension development with no effect on myosin light-chain phosphorylation or on signaling processes involved in the activation of the contractile apparatus (48). Furthermore, the degree of tension depression caused by the inhibition of actin polymerization is sensitive to the mechanical strain on the muscle, suggesting that actin polymerization is mechanosensitive (48). It is possible that the small fraction of actin undergoing polymerization resides in a cytoskeletal pool that is distinct from the actin that interacts with myosin (68, 69). This actin might function to regulate cell shape and rigidity and to connect the cytoskeletal lattice to the membrane to transmit force generated by the contractile apparatus. However, there is minimal information as to the function of the newly polymerized actin, how actin dynamics is regulated during contraction, how signals for the remodeling of actin might be coupled to receptor activation or mechanical stimulation, or how actin remodeling might be targeted to localized regions within the smooth muscle cell. Nonetheless, the sensitivity of actin dynamics to both mechanical and pharmacological stimuli suggests that the changes in the processes of actin dynamics throughout the contraction cycle represent an important component of the noise temperature. A rapid increase in the rate of actin polymerization after contractile stimulation would be expected to contribute to the high noise temperature during this phase of contraction.

Mechanical signals can be transduced and transmitted to the cytoskeletal system in smooth muscle by proteins that associate with transmembrane integrins in the membrane adhesion plaques of smooth muscle. Talin, paxillin, and focal adhesion kinase undergo phosphorylation during the contractile stimulation of airway smooth muscle (59, 84, 94). The phosphoryla-

tions of focal adhesion kinase and its substrate paxillin are sensitive to mechanical stimulation in smooth muscle cells and tissues (71, 72, 80, 82, 94). Both of these proteins play critical roles in the regulation of contraction in smooth muscle, and in the motility and migration of other cell types (28, 40, 56, 60, 83, 85). The depletion of either paxillin or focal adhesion kinase from airway smooth muscle tissues by treatment with antisense prevents tension development in response to a contractile stimulus (83, 85). Paxillin-depleted smooth muscle tissues exhibit normal increases in intracellular Ca^{2+} , myosin light-chain phosphorylation, and myosin ATPase activity in response to a contractile stimulus, but actin polymerization in response to contractile stimulation is inhibited (85). Hence, the suppression of tension development by paxillin depletion results from the disruption of cytoskeletal processes that are independent of cross-bridge cycling. What is more, the expression of a nonphosphorylatable paxillin mutant in smooth muscle tissues has a similar effect, indicating that not just the presence of paxillin but its phosphorylation is a critical event in the contractile response (86). In contrast, focal adhesion kinase is involved in regulating intracellular Ca^{2+} and thereby directly affects the activation of contractile proteins (83). These observations suggest a route by which external mechanical forces can directly and simultaneously impinge on the molecular processes involved in both cytoskeletal remodeling and the regulation of cross-bridge cycling. Thus the molecular processes involved in regulating cytoskeletal remodeling and cross-bridge cycling are tightly intertwined, and it is likely that the mechanical or pharmacological perturbation of either process will have an effect on the other.

Is there direct evidence for changes in the structural organization of the cytoskeletal lattice of smooth muscle cells during the contraction cycle? There is currently no data available that provide a direct visualization of the organization of actin filaments at different stages of contraction, doubtless because visualizing this in differentiated smooth muscle cells is a technically challenging feat. However, confocal images of tracheal smooth muscle cells dissociated from tissues reveal that vinculin and talin become more concentrated at the cell membrane in response to contractile stimulation (57). Both of these proteins are implicated in forming linkages between integrins and actin filaments; whereas talin can directly couple actin filaments to integrins, vinculin is believed to strengthen the attachments between actin filaments and integrins that are formed by other cytoskeletal proteins (10). These data imply that new links between actin and the integrin proteins are forming in stimulated smooth muscle. The recruitment of vinculin to the membrane is regulated by paxillin, thus providing a potential mechanism for the mechanosensitive regulation of vinculin recruitment during contraction (57). Because vinculin has been linked to the recruitment of the Arp2/3 complex in nonmuscle cells (13), the recruitment of vinculin might also catalyze the formation of new actin filaments. These observations most likely represent a

tiny slice of the cytoskeletal events that contribute to the transition of the smooth muscle cell from a malleable plastic state under resting conditions to a stiff relatively undeformable state during the tonic phase of contractile activation.

Clearly, much work will be required to elucidate the specific molecular events responsible for the remarkable mechanical properties of the smooth muscle cell. Indeed, it seems unlikely that any specific mechanical property can be attributed to any single molecular process. Even from the limited data currently available, it is clear that multiple interconnected molecular events are involved in determining the dynamics of cross-bridge cycling, the polymerization of actin, and the organization of the cytoskeletal lattice and that perturbation of any one of the various components of these processes is likely to significantly impact on the responses of another. Hence, changes in the noise temperature almost certainly reflect the integrated molecular events involved in regulating both cytoskeletal dynamics and cross-bridge cycling. But it is yet to be established whether analysis of the effective noise temperature will provide a useful probe of the kinetic status of these molecular events or the probability of molecular transitions within the cytoskeleton at any point during the contraction cycle.

MISSING THE BIG PICTURE?

As regards the glass hypothesis, the picture developed thus far is both incomplete and generic. The level at which events play out are not specified with any great precision, and it remains to be determined how the various molecular species described above may fit into the framework or fail to do so. But we are not left entirely in the dark. Whatever the role of individual species might be, the integrated effects are quite clear: measurements of the noise temperature during the contractile event demonstrate a rapid "heating," after which the system progressively "cools" (Fig. 1). At the same time, force and stiffness progressively increase and shortening velocity peaks rapidly and then progressively decreases, as does the rate of ATP utilization (Figs. 1–4).

Although the roles of other pertinent species are far from clear, these observations are consistent with what we already know about myosin cycling and actin polymerization in the airway smooth muscle cell. To see this, we recall that the noise temperature is composed of two components taken as a ratio (Fig. 3); it is the microscale agitation (numerator) taken relative to the height of energy barriers that tends to hinder hopping events (denominator). As such, there are two generic mechanisms by which the noise temperature can change. As regards the numerator, a variety of agencies might come into play, but among these it is easy to imagine the following. The initial transient of noise temperature (Fig. 1) is consistent with rapid myosin activation early in the contractile event and the onset of rapid myosin cycling. Associated agitation occurs within a cytoskeletal lattice that, at this early time, is

relatively dynamic. The early part of the activation transient is then followed by a progressive downregulation of cross-bridge cycling rates over a somewhat longer time scale as the muscle approaches the latch state through the action of myosin phosphatases (55). Similarly, in the denominator, it is easy to imagine that during the contractile event cytoskeletal elements become progressively polymerized and cross-linked (5, 37, 48), and, as they do, the depth of energy wells becomes progressively deeper and deeper. With the numerator decreasing and the denominator increasing, after the early part of the transient, the noise temperature can only fall. As the noise temperature falls, the rate of hopping must fall as well. But the hop is the elemental event for cytoskeletal rearrangement, with remodeling being the sum total of many such hopping events. As such, rapid downregulation of the noise temperature during the first minute of the contractile event would tend to freeze into the contractile response an indelible memory of events transpiring in its early history, such as those depicted in Fig. 2.

In conclusion, a somewhat loose but perhaps appropriate analogy is perhaps useful because it confronts us with a fundamental question. If the action potential is the overarching framework for understanding the diversity of ion channels and their individual roles in nerve dynamics, could it be that there is no corresponding framework for understanding the diversity of cytoskeletal molecules and their role in cytoskeletal dynamics? That is to say, are we to be left with answers only at the granular level in the scores of individual molecular species and a myriad details? The answer is, perhaps. If the glass hypothesis is eventually shown to be an inappropriate integrative framework, in its demise it might help to show that some other integrative framework is needed in its place.

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