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Remodeling of the airway smooth muscle cell: are we built of glass?

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Abstract

Classical understanding of airway lumen narrowing in asthma has held that the isometric force generated by airway smooth muscle (ASM) must be at every instant in a static mechanical equilibrium with the external load against which the muscle has shortened. It has been established recently, however, that this balance of static forces does not apply in the setting of tidal loading as occurs during breathing and must give way to the broader concepts of (1) the perturbed contractile state that exists far from static equilibrium conditions and (2) mechanical plasticity of the ASM cell. Here we describe the hypothesis that the well-established static contractile state, the newly-elaborated perturbed contractile state, as well as the remarkable mechanical plasticity of the ASM cell, are all subsumed under a rubric that is at once surprising, unifying and mechanistic. The specific hypothesis suggested is that the ASM cell behaves as a glassy material [Phys. Rev. Lett. 87 (2001) 148102]. A glass is a material that has the disordered molecular state of a liquid and, at the same time, the rigidity of a solid. If the hypothesis is true, then the ability of the ASM cytoskeleton (CSK) to deform, to flow and to remodel would be determined by an effective temperature—called the noise temperature—representing the level of jostling (i.e. molecular noise or agitation) present in the intracellular microenvironment. The abilities of the CSK to deform, to flow and to reorganize represent basic biological processes that underlie a variety of higher cell functions. If supported by the data, therefore, this integrative hypothesis might have implications in medicine and biology that go beyond the immediate issues of smooth muscle shortening and its role in asthma.

Keywords: Airway, lumen, smooth muscle; Disease, asthma; Muscle, smooth, reorganization; Plasticity, mechanical, airway smooth muscle

1. Introduction

To fashion a work of glass, a glassblower must heat the object, shape it, and then cool it down. Here we suggest the hypothesis that the airway smooth muscle (ASM) cell modulates its mechanical properties and remodels its internal cytoskeletal structures in much the same way. But instead of changing thermodynamic temperature, the cell is proposed to modulate an effective temperature—called the 'noise' temperature—representing the level of molecular agitation (noise)

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present in the intracellular microenvironment. This hypothesis leads to a series of testable predictions, including the prediction that the ASM cell should be able to remodel most rapidly when it is 'hot' but far less rapidly when it is 'cold'. These predictions and others are testable in large part because the noise temperature turns out to be both a measurable quantity and one that can be manipulated in a systematic fashion (Fabry et al., 2001). For example, we show below that the maximally activated ASM cell in isometric steady-state contraction is in a 'cold' state, whereas cells that are relaxed, or that are undergoing an activation transient, or that are fully activated but subjected to cyclic length changes, are all in relatively 'hotter' states.

A corollary hypothesis is that the ability of the ASM cytoskeleton (CSK) to deform, to flow, and to remodel its internal structures is determined by only a single parameter, namely, the noise temperature of the CSK matrix. If true, then the contribution of any specific molecular species to the integrated mechanical behavior of the matrix would be expressed mainly through its effects on the noise temperature. Although we have cast this integrative hypothesis within the particular context of airway hyperresponsiveness (AHR), it might well have implications in medicine and biology that go beyond the immediate issues of smooth muscle shortening and its role in asthma.

2. Airway hyperresponsiveness (AHR)

AHR 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). AHR is the basic feature that underlies the excessive airway narrowing that is characteristic of asthma, but its mechanism remains poorly understood. (King et al., 1999). Although asthma is usually defined as being an inflammatory disease, the link between the immunological phenotype and the resulting mechanical phenotype associated with disease presentation, including AHR, remains unclear; indeed, it is now established that AHR can be uncoupled from airway inflammation (Brusasco et al., 1998; Crimi et al., 1998; Holloway et al., 1999; Leckie et al., 2000; Drazen and Weiss, 2002). It remains equally unclear if AHR is due to fundamental changes in smooth muscle phenotype, plastic remodeling of ASM cytoskeletal architecture, structural and/or mechanical changes in the non-contractile elements of the airway wall, or alterations in the coupling of the airway wall to the surrounding lung parenchyma (King et al., 1999; Milanese et al., 2001).

3. The static universe and its failure to explain AHR

To explain AHR, the classical theory of airway lumen narrowing has held that the isometric steady-steady active force generated by ASM must be at every instant in a static mechanical equilibrium with the external load against which the muscle has shortened (Macklem, 1996; Lambert et al., 1993). Accordingly, research aimed at elucidating AHR tended to emphasize those two factors and the conditions that cause them to change. It is now firmly established, however, that this balance of static forces, no matter how elaborate the underlying analysis might be, in the end fails to explain airway narrowing in the dynamic setting of tidal loading as occurs during breathing or following deep inspirations (Fredberg, 2000b). Indeed, notions of statically equilibrated systems have to fail because they do not encompass major determinants of airway narrowing that are now known to be dynamically equilibrated, including plastic adaptations of the ASM CSK and perturbed contractile states that exist far from static equilibrium conditions. These dynamically equilibrated phenomena and their relationship to airways hyperresponsiveness in asthma are described in several recent reviews (Seow and Fredberg, 2001; King et al., 1999; Fredberg, 2000b). Here and in the remainder of this article we use the term 'CSK' in the broadest sense so as to subsume scaffolding proteins, the contractile apparatus and any attached structures or molecules that contribute appreciably to the integrated mechanical properties of the cell.

As regards plastic adaptation of the CSK, it has been demonstrated recently that over a remarkably short period of time the ASM cell has the ability to adapt its optimal length (L_0) over an impressively wide range, approaching 3-fold changes of L_0 in the same muscle within a time frame of 1 h or less (Kuo et al., 2001; Pratusevich et al., 1995; Wang et al., 2001; Gunst and Wu, 2001; Gunst et al., 1995). In particular, it has been suggested that progressive plastic adaptations of ASM to shorter working lengths may play an important role in AHR. Remodeling of the CSK is a never-ending process, and its rate seems to be actively modulated by the cell, with the actin lattice, the myosin filament and the focal adhesion plaque all being evanescent structures that can be virtually demolished in some circumstances and then reconfigured and stabilized in others (An et al., 2002; Mehta and Gunst, 1999; Seow et al., 2000; Balaban et al., 2001; Kuo et al., 2001; Seow and Fredberg, 2001). Moreover, plasticity of the CSK lattice and perturbed contractile states have the ability to interact (Fredberg, 2000a; King et al., 1999).

4. Cells and soft glasses: strange bedfellows

The hypothesis addressed here is that the well-established static contractile state, the newly-elaborated perturbed contractile state, as well as the remarkable plastic adaptations/remodeling of ASM cells that have been recently reported (Fig. 1), are all subsumed by glassy behavior and, in particular, the behavior of soft glasses. We have recently established evidence, described below, that is strongly suggestive that at least five cell types in culture, including the ASM cell, belong to this class (Fabry et al., 2001). Surprisingly, other substances within the class include foams, emulsions, colloid suspensions, pastes and slurries (Sollich, 1998; Sollich et al., 1997). Existing models of viscoelasticity have thus far failed to provide a satisfactory explanation of the mechanical behavior of these systems.

There are three empirical criteria that define the special class of soft glassy materials (Sollich, 1998; Sollich et al., 1997). The first of these is that they are soft; the Young's modulus of these materials (Stamenovic and Coughlin, 1999) is smaller than the softest of man-made polymers or rubbers by several orders of magnitude (Fig. 2). The second criterion that defines the class is that their dynamics is 'scale-free' (Sollich, 1998), meaning that when matrix stiffness is measured over a wide range of frequencies, no special frequency, molecular relaxation time, or resonant frequency stands out. Instead, the stiffness increases with frequency, f, in a featureless fashion following a weak power law, f^{x-1}. As we demonstrate below, x can be interpreted as a noise temperature, which is easily quantified from the observed power law exponent and is found to lie close to but slightly larger than unity. Scale-free behavior stands in contrast with the frequency-dependent stiffness of common polymer matrices, whose spectra typically display distinct plateaus, shoulders and inflections; these features are indicative of a transition from one dominant mechanism to another (Ward, 1983). Rubbers, for example, show a transition from dominance of chain entropy at lower frequencies, where stiffness falls in the MPa range, to dominance of van der Waals bonds at higher frequencies, where stiffness falls in the GPa range. Such frequency-dependent transitions do not occur in scale-free media, in which case molecular relaxation processes in the matrix are not tied to any particular internal time scale or any distinct molecular rate process.

The third criterion that defines the class of soft glassy materials is that the dominant frictional stress seems not to be of a viscous origin or attributable to a viscous-like stress. Instead, the frictional stress in these materials is found to be proportional to the elastic stress, with the constant of proportionality, the hysteresivity η, being of the order 0.1 (Sollich, 1998; Fredberg and Stamenovic, 1989). That is to say, frictional stress within the matrix is much smaller than elastic stress, and when these stresses change they always do so in concert. Thus, like elastic stresses, frictional stresses are found to be scale-free and to increase with frequency with the same weak power law.

Fabry et al. (2001) have offered clear evidence that five cell types in culture, including the ASM cell, satisfy all of these empirical criteria, and

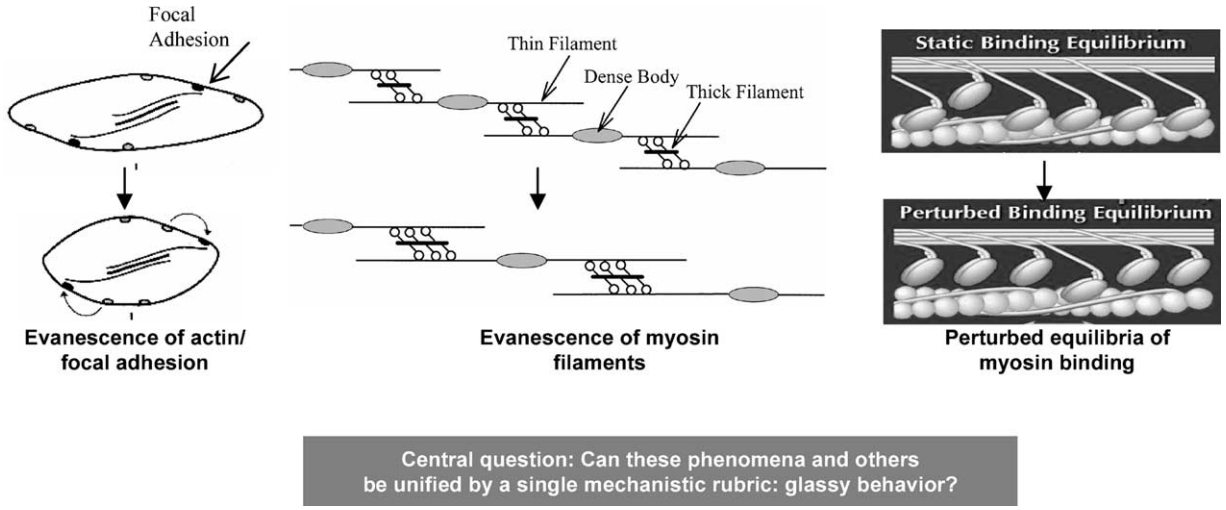

Fig. 1. The end-effector of acute airway narrowing in asthma is ASM. It was thought until recently that the role of ASM in airway narrowing was functionally specified by its degree of activation and its static force-length curve. To the contrary, we now know that even modest agitation, such as length changes as occur during breathing, profoundly perturbs the binding of myosin to actin and plastically remodels the CSK lattice; the contractile state is dynamically equilibrated (right) and the associated cytoskeletal scaffolding is evanescent, being rapidly demolished in some circumstances and reconstructed in others (left and middle). To explain airways hyperresponsiveness, phenomena such as those depicted below have been suggested, as have others including the failure of deep inspirations to dilate the asthmatic but not the normal airway (Skloot et al., 1995), latch (Fredberg et al., 1996), and the role of specific CSK molecules in muscle plasticity(Seow and Fredberg, 2001; Halayko and Solway, 2001). Nonetheless, these phenomena would seem to be mostly unconnected from each other and remain largely unexplained. Each mechanism that has been proposed addresses only a subset of the questions necessary for a comprehensive theory, and all contain large explanatory gaps. This constellation of diverse findings and multiple potential mechanisms may drop into a unified pattern, however, when the ASM cell is thought of as being a glassy material.

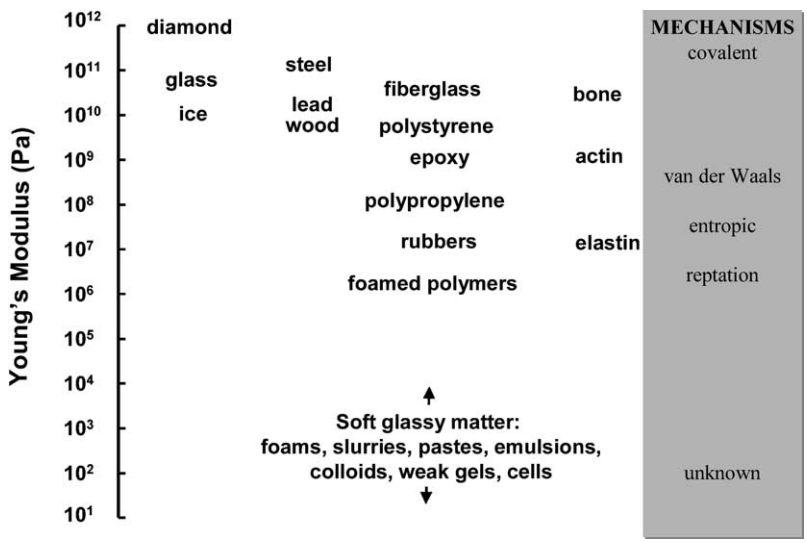

Fig. 2. The stiffness of common materials spans an exceedingly wide range, with living cells and other soft glasses at the lowest end of this range. Mechanisms accounting for matrix stiffness are shown at the right.

therefore, belong to this special class of materials. Reconstituted biopolymer model systems, however, as distinct from the living cell, typically show distinct time constants, are not scale free, and seem not to fall within the class (Schnurr et al., 1997; Goldmann et al., 1997; Gisler and Weitz, 1999; Ziemann et al., 1994), although in some special cases, such as F-actin networks cross-linked with filamin and α-actinin, behavior approximating a weak power law has been reported (Tseng and Wirtz, 2001; Goldmann et al., 1997).

Each of these soft glassy systems—foams, emulsions, pastes, slurries, colloid suspensions and living cells—is comprised of molecules and microstructures that differ rather dramatically from one another. Nonetheless, their mechanical properties are found to be governed by the very same empirical law (called the structural damping law (Fredberg and Stamenovic, 1989; Sollich, 1998; Fabry et al., 2001). The key question then becomes, How can such diverse systems express mechanical behavior that is so much alike? Based on the diversity of system specifics, Sollich suggested that the defining mechanical features of this class must be not so much a reflection of particular molecules or molecular mechanisms and instead might be a consequence of a generic system property at some higher level of structural organization (Sollich, 1998).

5. Messy mechanical systems

A glass is a material that has the disordered molecular state of a liquid and, at the same time, the rigidity of a solid (Torquato, 2000). Folklore has it that ordinary window glass would appear to be a solid material when viewed on human time scales, yet is all the time slowly flowing; tour guides, for example, often point out that the window glass of old European cathedrals is thicker at the bottom than the top, and they explain that this is so because the glass has been flowing under the influence of gravity for many centuries. This interpretation is still debated among physicists, however, (Zanotto and Gupta, 1999). Indeed, 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 at an intersection of open questions in disciplines previously thought of as being distinct.

Although glassy matter is poorly understood, current literature suggests the following. A glass arises in any liquid that solidifies too fast for structural elements to form an ordered array and a corresponding solid state, as would any ordinary solid (Torquato, 2000; Bouchaud, 1992; Liu and Nagel, 1998; Weeks et al., 2000; Weitz, 2001; Ediger, 2000; Durian and Diamant, 2001). Even though the state of least free energy would be ordered, 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. 3). In that cage (equivalently, an energy well), elements are trapped away from energy minima, and therefore, the system is not at a thermodynamic equilibrium.

Jostling caused by thermal agitation can cause a microscale element to hop out of its current cage and fall into a nearby cage (Fig. 3). Thus, each element does not vibrate about a fixed spatial address as it would in an ordinary solid matrix. Rather, the existence of ongoing hopping events implies that the links between neighboring elements are impermanent (metastable) and that the elements are constantly rearranging themselves in a never-ending search for order. They find themselves kinetically stable enough to exhibit the structural rigidity of a solid, yet they are not truly thermodynamically stable. It follows logically that if individual elements can hop, then the matrix as a whole can flow, reorganize internal structures and become internally disordered (Fig. 3). That is to say, at the molecular level a glass is by definition both a disordered mechanical system and one that is in a continuous state of remodeling. However, if the temperature is decreased then thermal energy becomes ever less available, the weak bonds between elements constrain the motion more and more, and the rate of hopping slows.

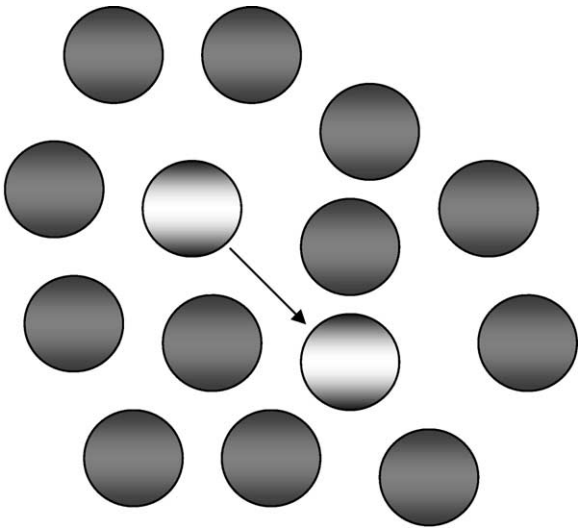

Fig. 3. Cardinal features that define glassy matter are:

- Interactions between elements are too complex and too weak to form ordered structures spontaneously.
- Each element is trapped in a cage formed by its neighbors.
- Microscale agitation can cause the element to hop out of its current cage and fall into a nearby cage.

Taken together, these features implicate metastability and disorder as being key determinants of the ability of any glassy matrix to deform, flow or reorganize.

With application of a stress that is sufficiently large window glass will fracture. A soft glass, however, will merely flow. Nonetheless, the special class of soft glasses has much in common with ordinary glasses. According to the theory of Sollich, the ability of an element in a soft glass to escape its cage is not dominated by thermal agitation, as in window glass, but rather by an effective temperature that represents the amount of non-thermal jostling (i.e. the molecular noise, or agitation, addressed below). The population of stress bearing elements becomes distributed among energy wells, or traps, of depth E, and the rate at which elements hop out of a well is proportional to $e^{-E/x}$. In Sollich's theory the factor x is identified as the effective temperature, or the 'noise' temperature of the matrix, where x is seen to replace the thermal energy kT in the familiar Boltzmann exponential. In order to take into account heterogeneity of the matrix, energy well depth E is reasoned to be distributed broadly. To escape its cage, an element in a soft glass must get kicked over energy barriers that are large compared with thermal energies. But if the noise temperature is reduced enough that hopping virtually ceases, then individual elements become trapped in place, the matrix can no longer flow or reorganize, and the material becomes a simple elastic solid; this is called the glass transition. If the jostling energy is expressed relative to the depth of the energy well, then the glass transition is approached as x approaches unity (Sollich, 1998; Sollich et al., 1997).

If thermal energy is insufficient, what then is the source of jostling that might activate microscale hopping event? Sollich imagined a structural element in a soft glassy matrix to be agitated by its mutual interactions within neighboring elements in the immediate microenvironment. That is to say, random agitation (noise) can excite a metastable element causing it to hop, in turn triggering secondary rearrangements and hopping events that ripple through the system (Weitz, 2001). But any more precise notion of the agitation mechanism remains to be identified, and this gap of understanding is serious because it lies at the center of Sollich's theory. Ironically, this ambiguity may resolve spontaneously in the case of living cells. As opposed to the case of inert materials for which the theory was originally devised, there exists an obvious and ready source of non-thermal energy that is being injected into the CSK matrix all the time, namely, those proteins that go through cyclic conformational changes and thus provide an ongoing source of microscale agitation. It is plausible, therefore, that such events might contribute to the noise microenvironment by mechanisms that are ATP-dependent (Fig. 4). But whether such processes set the noise temperature of the living cell and, if so, which proteins may dominate the agitation, are not yet established experimentally.

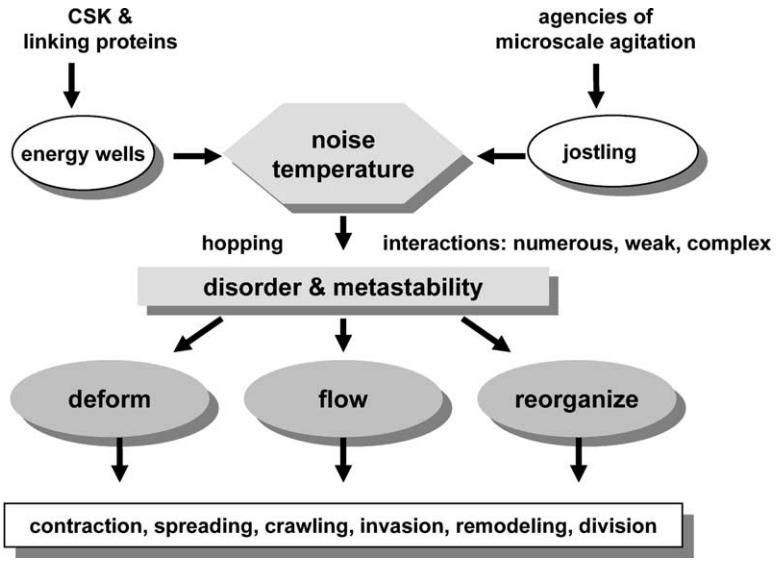

Fig. 4. Instead of changing temperature, the cell is proposed to modulate its mechanical properties by changing an effective temperature, called the 'noise' temperature. The noise temperature is a measure of the amount of jostling (agitation) present in the intracellular microenvironment relative to the depth of energy wells that constrain rearrangements of structural elements. Since it is the jostling energy relative to energy well depth, the noise temperature as used here is dimensionless. Jostling: In addition to ordinary thermal agitation (kT) and the tidal action of spontaneous breathing, other agencies potentially contributing to the intracellular agitation include vesicle transport and any local motions caused by proteins that undergo cyclic conformational changes, including molecular motors, ribosomes and G-proteins. These ongoing conformational changes jostle nearby cages by mechanisms that are ATP-dependent. Local motions induced by conformational changes are in the 1–10 nm range (Howard, 2001), which is comparable to molecular dimensions and molecular spacing. The mechanical energy released is also quite large, about half of the chemical energy derived from hydrolysis of the gamma phosphate bond of ATP, or roughly 10 kT per event (Howard, 2001). For a typical cell these events occur frequently, at a rate of 10^7 per sec. Energy wells: Energy wells that constrain motion are imagined to deepen with the buildup of weak bonds and geometrical constraints associated with CSK polymerization and crosslinking. Deepening of such wells would be reflected in a falling noise temperature. Disorder and Metastability: The glass hypothesis rests on only very conservative assumptions, namely, that the CSK is comprised of elements aggregated with one another via interactions that are numerous, weak and complex. As a result, these interactions are metastable and associated stochastic hopping events lead to lattice disorder. Physiologic Endpoints: An attractive feature of the glass hypothesis is its explanatory power; it attempts to explain with a single unifying mechanism three aspects of CSK function that have been investigated previously as largely distinct and independent events-deformability, flow and remodeling.

6. Are we built of glass?

A majority of our body weight is attributable to bone, connective tissues and striated muscle, and for these tissues the notion of glassy behavior is almost surely inapplicable. Nonetheless, that leaves a wide variety of cell types whose mechanical properties are not well understood. Identification of the living cell in culture as belonging to the class of glassy matter at first surprised us, but on further analysis seemed mechanistically plausible. In the living cell the energy barriers that hold elements in place are likely to be the weak bonds or steric constraints that arise during polymerization of cytoskeletal structures. Moreover, interventions associated with formation of these weak bonds tend to push the system toward its glass transition and are reflected by a falling noise temperature (Fig. 5). Indeed, our data suggest that the living cell exists quite close to a glass transition and modulates its mechanical properties by moving between glassy states that are 'hot', melted and liquid-like and states that are 'cold', frozen and solid-like (Fig. 5). More than a super-

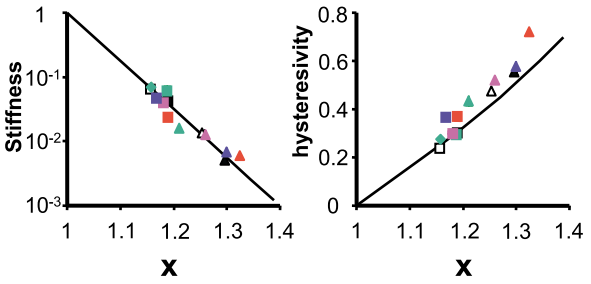

Fig. 5. As the noise temperature falls cytoskeletal stiffness increases and hysteresivity falls, and conversely. In the limit that the noise temperature approaches unity, matrix stiffness approaches a maximum, and hysteresivity approaches zero, thus approximating a frozen state. This is called the glass transition. Adapted from Fabry et al. (2001). Remarkably, data for five cell types and multiple interventions collapse onto two master scaling curves, one describing elasticity and the other friction. Therefore, the mechanical changes caused by specific interventions, and the specific molecules that they affect, are explained solely by the change in noise temperature that they engender. As such, mechanics of the integrated matrix would appear to express a colligative quality. Symbol Key: human ASM cells (black), human bronchial epithelial cells (blue), mouse embryonic carcinoma cells (F9) cells (pink), mouse macrophages (J774A.1) (red) and human neutrophils (green) under control conditions (■), treatment with histamine (□), FMLP (◆), DBcAMP (△) and cytoD (▲). Black solid lines are predictions (not a fit to the data) from the structural damping equation.

ficial metaphor, this is a precise mechanistic statement of the glass hypothesis and has implications that are quantitative and testable (Table 1).

As are other soft glasses, the cell is a 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 (Fig. 6); it is, after all, its disordered amorphous structure that gives 'smooth' muscle its name. This evidence is entirely qualitative, however, and as regards cell mechanical functions described in the literature it is quite clear that CSK disorder is a question that has been actively avoided. Thus, the primary practical implication of the glass hypothesis is that the extent of disorder of the cytoskeletal matrix, as well as metastability of its structural elements, now come to the forefront as two central features that control cytoskeletal mechanics.

While quite disordered compared with striated muscle cells, smooth muscle cells are certainly not without some degree of systematic structural organization (Small and Gimona, 1998). In the context of glassy behavior of the CSK, then, what do we mean by the notion of structural disorder? Sollich offers only a functional answer to the question (Sollich, 1998). He argues that structural

Table 1
The glass hypothesis leads to quantitative, mechanistic testable predictions

(1) Response to small imposed deformations (either step or cyclic at frequency f)
  Time-scale invariance: dynamic responses of CSK are not tied to any particular time scale
    CSK stiffness must follow a simple power law f^{a-1}
    CSK internal friction also must follow a simple power law f^{b-1}
    displacement (creep) in response to a small step force must decay as the power law, t^{c-1}
    stress in response to a small step displacement must relax as the power law t^{1-d}
  Structural damping
    elastic and loss moduli must conform the structural damping law (Fabry et al., 2001)
    power law exponents must be equal; a = b = c = d = x = noise temperature
(2) Specific molecular interactions contribute to aggregate mechanical behavior only to the extent that they can alter x
    System stiffness determined solely by x
    System friction (hysteresivity η) determined solely by x
(3) Response to a step deformation of strain amplitude γ
For small γ, CSK stress does not decay in time as an exponential process, but rather as a power law
For large γ, relaxation processes speeded by a factor $\exp(\gamma^2/2x)$
(4) CSK remodeling: rate at which the CSK reorganizes its internal structures exhibits a precipitous slowdown as x falls
(5) ATP-dependent processes might contribute appreciably to microscale agitation and the noise temperature

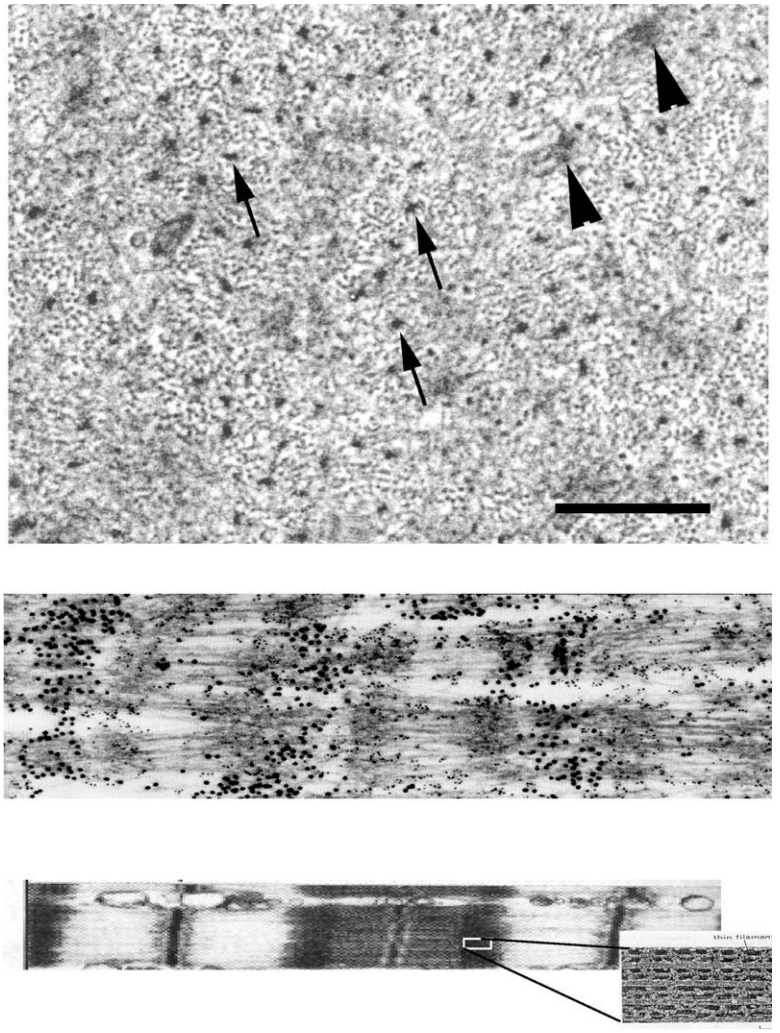

Fig. 6. Order within the cytoskeletal matrix is a matter of degree. Examples of disordered, partially ordered and highly ordered matrices. Top: pig ASM cell in transverse section shows distribution of dense bodies (arrowheads) and myosin thick filaments (arrows) that is very disordered. Scale: 200 nm. (TEM image kindly provided by C. Seow). Middle: A fibroblast in longitudinal section shows a contractile apparatus that is partially ordered (adapted from Alberts et al., 1989). Bottom: Striated muscle shows a contractile apparatus that is well-ordered.

disorder can be expressed by its functional consequences, namely, by wide distributions of both local microscale strains and energy well depths. In addition, the hopping event implies ongoing microstructural rearrangement, shuffling the deck as it were, giving yet another notion of disorder. In the theory of soft glassy materials, the notion of disorder, while being central, remains an elusive concept. We will return to the issue of disorder below.

7. Limits of reductionism?

Metastability of weak interactions between elements and resulting disorder are the hallmarks of

glassy systems (Sollich, 1998; Sollich et al., 1997). And, like any glass, cells are widely recognized as existing far from thermodynamic equilibrium (Alberts et al., 1989). For a disordered nonequilibrium system to modulate its mechanical properties in an orchestrated manner, as the cell must do during routine mechanical functions including contraction, spreading, crawling, invasion, remodeling or division, glassy behavior would appear to be a logical solution and perhaps even a biological imperative. Nonetheless, the fundamental link between variations of CSK disorder and metastability on the one hand and changes of cell mechanical properties on the other had until now escaped attention.

The consequences of these ideas are interesting. As opposed to focusing attention on details of specific molecular events, such as the acto-myosin interaction, those associated with the Rho family of proteins, or myosin phosphorylation (Halayko and Solway, 2001), the glass hypothesis focuses attention instead on a higher level of structural organization. This is not meant to imply that molecular details are unimportant; to the contrary, there are many individual proteins whose inhibition or dysfunction has catastrophic consequences and whose molecular pathways are critically important. Nonetheless, glassy behavior suggests that some of the important molecular interactions may contribute to cytoskeletal dynamics and remodeling mainly to the extent that they are able to modulate the noise temperature. As such, the glass hypothesis subordinates the role of individual molecular species and puts them instead into a nonspecific but deeply integrative context (Figs. 4 and 5). This point of view is novel in that it implies a colligative quality of the contribution of individual molecular species to the noise temperature and associated mechanical behaviors of the integrated matrix, and in that regard it is reminiscent of the contribution of arbitrary solutes to the osmotic stress and associated fluxes of solvent. On the one hand, this implies inherent limits to reductionism, where by reductionism we mean the belief that the properties of a system are wholly explainable in terms of properties of system constituents at some lower level. As Lewontin has argued, in some systems to study specific molecular constituents in isolation might be to destroy the very interactions that need to be studied (Lewontin, 2000). On the other hand, to elucidate a particular molecular-level event in situ without regard to its impact on the noise temperature might be to miss a major facet of its function.

8. Explaining mechanical plasticity

The contractile state of smooth muscle in steady-state isometric conditions is called the latch state. It is known to be a cold/frozen contractile state characterized by slowing of crossbridge cycling rates and the rate of ATP hydrolysis (Murphy, 1994; Fredberg et al., 1996). The hysteresivity η of ASM is a measure of internal mechanical friction and is closely coupled to the rate of crossbridge cycling as reflected both in the unloaded shortening velocity and the rate of ATP utilization measured by NADH fluorimetry (Fredberg et al., 1996). The progressive fall of η after contractile stimulus onset (Fig. 7) has been interpreted as reflecting rapidly cycling crossbridges early in the contractile event converting to slowly cycling latch bridges later in the contractile event (Fredberg et al., 1996). This molecular picture fits well with computational analysis based on first principles of myosin binding dynamics and their disruption by imposed muscle stretches (Mijailovich et al., 2000; Fredberg, 2000a), which suggests further that these perturbed states are closely related to the Fenn effect during constant velocity shortening.

A rather different perspective on these observations, and one bearing on CSK plasticity, arises when they are viewed instead through the lens of glassy behavior. We note, first, the fact that the hysteresivity of a soft glass is linked to the noise temperature x by the simple relationship $\eta = \tan \pi(x-1)/2$ (Fabry et al., 2001). This relationship implies a simple proportionality between η and x when x is close to unity, as it is within the physiological range (Fig. 5). Accordingly, in isometric conditions early in the activation transient the muscle is seen to be 'hot' but then becomes gradually 'colder' as it approaches steady-state contraction and the latch state (Fig. 7). The glass

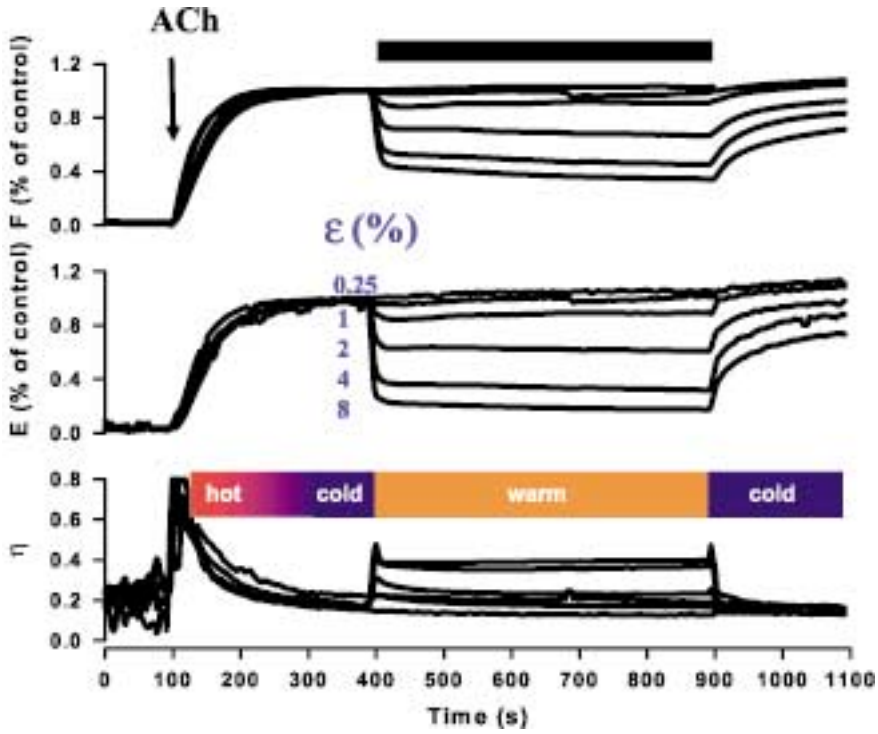

Fig. 7. Isometric contraction of a bovine ASM strip (10^{-4} M acetylcholine at 100 sec) showing muscle force F, stiffness E and hysteresivity η; ε is the amplitude of cyclic stretches expressed as a percent of muscle length. In the range shown, η is proportional to the noise temperature x (Fabry et al., 2001). Therefore, the system is seen to be 'hot' early in the contractile event (red) but progressively cools as the muscle enters the latch state (blue). Data adapted from (Fredberg et al., 1997) imposition of tidal oscillations of muscle length (400–900 sec, denoted by black overbar), simulating the action of quiet tidal breathing, causes force and stiffness to fall quickly and in a graded manner depending on stretch amplitude, but hysteresivity to increase. This observation is consistent with the interpretation that imposed macroscale agitation increases jostling of stress-bearing elements at the microscale as reflected by an increased noise temperature (orange). When the tidal stretches are terminated (900 sec), the noise temperature (reflected by η)?? suddenly falls. Remodeling events transpiring as the muscle adapts to imposed length oscillations become trapped-frozen as it were-for the duration of the contractile event. The glass hypothesis suggests, then, that the matrix is most adaptable when it is 'hot', and that these adaptations become fixed when the matrix is quenched by a noise temperature that is suddenly lowered.

hypothesis predicts, therefore, that the cell ought to be able to adapt faster to step length changes imposed while the cell is 'hot' (i.e. early in activation), and far less so while it is 'cold' (i.e. latch). If true, then transient elevation of the noise temperature early in the contractile event is consistent with, and might help to explain, the observation of Gunst and colleagues (Gunst et al., 1995), who showed that an imposed step change of muscle length alters the level of the subsequent force plateau to a degree that depends mostly on the timing of the length change with respect to stimulus onset (Fig. 8). Similarly, it is consistent with the findings that repetitive activation and deactivation substantially accelerates the rate of plastic adaptation of muscle to a change of its operating length (Pratusevich et al., 1995; Wang et al., 2001).

Changes of the noise temperature also help to explain a nagging loose end from our own laboratory (Fredberg et al., 1997). We had reported that during tidal loading of activated ASM, simulating the action of breathing, the greater is the imposed tidal stretch amplitude, the smaller are the subsequent plateaus of force and stiffness after tidal stretches are terminated, suggesting a

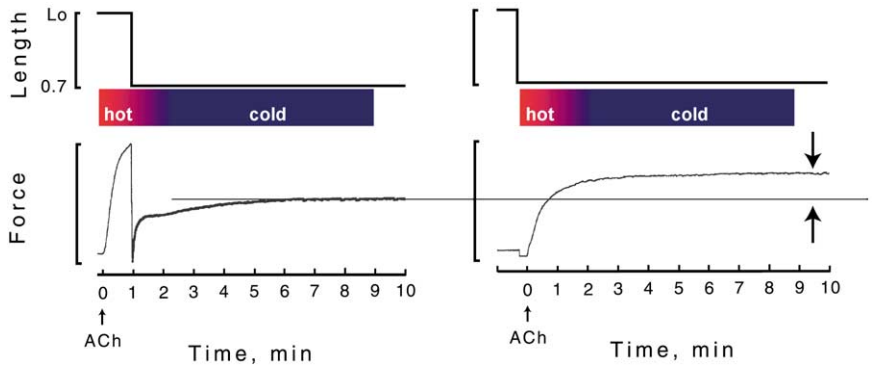

Fig. 8. Effect of a sudden length change imposed after smooth muscle has been activated by a contractile stimulus (left). Because the CSK matrix has substantially 'cooled' before the length is changed, the muscle cannot effectively adapt to its new length and thus produces only a relatively small asymptotic force. By contrast, when a sudden length change is imposed immediately before contractile activation (right), subsequent adaptation to the new length transpires while the matrix is relatively 'hot' and, according to the hypothesis, might account for the larger asymptotic force at the same length. Whatever adaptation events might have transpired, they must have been rapid, and faster in the hot state than in the cold state. Data adapted from (Gunst et al., 1995); these investigators did not measure hysteresivity or noise temperature in their studies; color changes are inferred by comparison with Fig. 7, which used a similar experimental preparation.

long lasting adaptation of the muscle to the prior cyclic load (Fig. 7). We now speculate that these plastic adaptations might be explained if these cyclic deformations imposed at the macroscale were to add to the agitation already present at the microscale and thereby increase the noise temperature in the CSK lattice, as is seemingly reflected by the observed stretch-induced increases of η (Fig. 7). Stretch-induced increase in noise temperature predicts a speeding up of internal molecular dynamics, including accelerated plastic restructuring events as the CSK adapts to its cyclic load. When the tidal stretches are terminated ($t >$ 900 sec, Fig. 7), however, the noise temperature (again, reflected by η) is suddenly lowered and these plastic changes might become trapped-frozen as it were-for the duration of the contractile event.

Changes in ultrastructure bear directly on this interpretation. Kuo and colleagues have reported that imposition of oscillatory mechanical strain causes myosin filaments to depolymerize, fragment and shorten (Kuo et al., 2001). Based on these observations they suggested that both oscillatory and step changes of muscle length tend to induce cytoskeletal disorganization. This induced disorganization, they argued, represents the first step of the remodeling process. During subsequent isometric contraction, myosin filaments then polymerize, defragment, reorganize and lengthen. These structural changes are very much in keeping with the hypothesis of glassy behavior which, potentially, sets these observations into a broader context.

The glass hypothesis suggests, then, that the matrix is most adaptable when it is 'hot', and that these adaptations become relatively fixed when the matrix is quenched by a noise temperature that is suddenly lowered. Such an interpretation would seem to bear not only on the issues of muscle plasticity, but also the bronchoprotective and bronchodilatory effects of deep inspirations (Skloot et al., 1995; Scichilone et al., 2001). Logically, of course, it must be specific molecular interactions that define the operative constituents of the cytoskeletal glass, their interactions and, therefore, the nature of the energy wells that determine the noise temperature. Accordingly, the individual molecular mechanisms of plasticity as described in the laboratories of Gunst, Seow and Ford are not inconsistent with this integrative point of view of the CSK as a glassy material, and may well fit within it (Fig. 4).

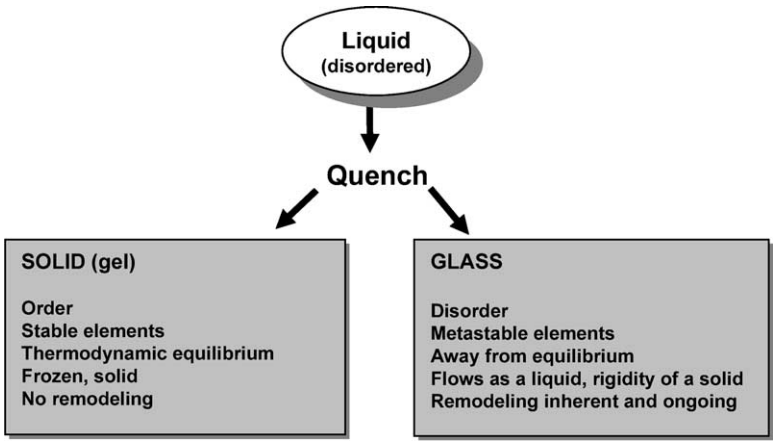

Fig. 9. The liquid-like cytoplasm can solidify in one of two ways, each with different mechanical implications. Mainstream thinking has postulated the gelation pathway. Alternatively, here we have explored the possibility that the matrix might solidify as a glass. A quenching event in the case of the ASM cell might correspond to exposure to a contractile agonist with ensuing polymerization of the actin lattice and activation of contractile proteins (Mehta and Gunst, 1999; An et al., 2002).

9. Cell rheology: mainstream hypotheses

Here we digress briefly to juxtapose the glass hypothesis against prevailing mainstream ideas concerning cell rheology (Fig. 9). As opposed to a glass, most often the CSK matrix has been thought of as being a gel at thermodynamic equilibrium (Pollack, 2001; Stossel, 1982; Janmey et al., 1994). If the CSK were simply a gel-like elastic body, it would maintain its structural integrity by developing internal elastic stresses to counterbalance whatever force fields it might be subject to. However, those same elastic stresses would tend to oppose-or even preclude altogether-other essential mechanical functions such as cell spreading, crawling, extravasation, invasion, division and contraction, all of which require the cell to ''flow'' like a liquid. A liquid-like CSK, however, would be unable to maintain its structural integrity. The classical resolution of this paradox has been the idea that cytoskeletal polymers go through a sol-gel transition, allowing the CSK to be fluid-like in some circumstances (the sol phase) and solid-like in others (the gel phase). The glass hypothesis holds that rather than being thought of as either a gel or a sol, the CSK may be thought of more properly as a glassy material existing close to a glass transition, with mechanical properties changing smoothly and continuously between liquid-like and solid-like states as governed by the local noise temperature (Fig. 5). A decreasing noise temperature is consistent with the formation of structure, which in turn may be associated with the notion of de Gennes (1979) that chemical bonding is equivalent to attractive interactions; as x decreases the system becomes more ordered and approaches a solid-like frozen state. Conversely, relaxing agonists and agents that disrupt the CSK cause x to increase and cause the system to become more disordered and move towards a fluid-like state (Fig. 5).

In that connection, it is important to recognize that soft glassy behavior stands in contrast with another prevailing paradigm of cell mechanics, which holds that cell mechanical properties arise from an interaction of distinct elastic and viscous components, and therefore, express only a limited range of characteristic relaxation times (Zahalak et al., 1990; Hochmuth, 2000; Evans and Yeung, 1989). Viscoelastic models with a sufficiently wide distribution of relaxation times can be forced to fit any realizable dependence of stiffness and loss moduli on frequency, but in the absence of clearly established mechanism such curve fitting procedures offer no insights as to mechanism and do not broach the further issues of remodeling and plasticity, leaving them as explanatory gaps. The glass hypothesis, by contrast, holds that elasticity,

friction and remodeling cannot be attributed to separate structural components or distinct processes (Sollich, 1998; Fredberg and Stamenovic, 1989). Instead, it proposes that the elastic stress, the frictional stress and the remodeling process share the very same locus, namely, energy wells associated with formed cytoskeletal structures, and that the unifying event becomes the hop from one energy well to another. The hop links friction directly to elasticity because energy dissipated is precisely the elastic strain energy that had been stored (Fredberg and Stamenovic, 1989; Sollich, 1998). Moreover, the hop itself is seen as being the elemental remodeling event.

10. Concluding remarks

We have put forward here the hypothesis that the behavior of soft glasses, and the underlying notion of the noise temperature, might provide a unifying explanation of the ability of the cytoskeletal lattice to deform, to flow and to remodel (Fig. 4). Sollich proposed that the common mechanical properties that all soft glassy materials share derive from generic features: structural elements that are discrete, numerous, aggregated with one another via weak interactions, and arrayed in a geometry that is structurally disordered and metastable. We have proposed that these features may comprise the basis of CSK rheology and remodeling. Because this hypothesis leads to a series of testable predictions (Table 1), in the coming years we may be able to develop a better idea of the extent to which these ideas can or cannot help to explain a variety of mechanical cell functions.

Although the glass hypothesis may seem to represent a rather sharp point of departure from current mainstream thinking in cell mechanics, it was anticipated more than 50 years ago by (Crick and Hughes (1950), who said,

> If we were compelled to suggest a model (of cell rheology), we would propose Mother's Work Basket—a jumble of beads and buttons of all shapes and sizes, with pins and threads for good measure, all jostling about and held together by colloidal forces.

As a qualitative definition of a soft glass, theirs is as good as any.

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