Available online at www.sciencedirect.com

ScienceDirect

Physics of Life Reviews ••• (••••) •••–•••

PHYSICS of LIFE reviews

www.elsevier.com/locate/plrev

Reply to comment

Energetics: An emerging frontier in cellular mechanosensing Reply to comments on "Cellular mechanosensing of the biophysical microenvironment: A review of mathematical models of biophysical regulation of cell responses"

Bo Cheng [a,b,1], Min Lin [a,b,1], Guoyou Huang [a,b], Yuhui Li [a,b], Baohua Ji [c], Guy M. Genin [a,b,d], Vikram S. Deshpande [e], Tian Jian Lu [f], Feng Xu [a,b,*]

[a] *The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, PR China*

[b] *Bioinspired Engineering and Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an 710049, PR China*

[c] *Biomechanics and Biomaterials Laboratory, Department of Applied Mechanics, Beijing Institute of Technology, Beijing, PR China*

[d] *Department of Mechanical Engineering & Materials Science, and NSF Science and Technology Center for Engineering Mechanobiology, Washington University in St. Louis, St. Louis 63130, MO, USA*

[e] *Department of Engineering, University of Cambridge, Cambridge CB2 1PZ, United Kingdom*

[f] *MOE Key Laboratory of Multifunctional Materials and Structures, Xi'an Jiaotong University, Xi'an 710049, PR China*

Received 20 September 2017; accepted 20 September 2017

Communicated by E. Di Mauro

Abstract

How do cells can sense the substrate stiffness? Our recent review highlighted a range of theoretical models and simulations that have been proposed to answer this important question. In response to this review, three leading groups in the field noted some important omissions not only from our review itself but also from the field. These groups noted, correctly, that much of our understanding of cellular mechanosensing arises from models that take advantage of equilibrium thermodynamics, and that this is inappropriate because living cells are never in thermodynamic equilibrium. In this response, we highlight some promising research aimed at resolving this conundrum.

Keywords: Molecular clutch model; Stiffness sensing; Mechanobiology

DOI of original article: https://doi.org/10.1016/j.plrev.2017.06.016.

DOIs of comments: https://doi.org/10.1016/j.plrev.2017.08.013, https://doi.org/10.1016/j.plrev.2017.07.004.

* Corresponding author at: The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, PR China.

E-mail address: fengxu@mail.xjtu.edu.cn (F. Xu).

[1] Authors contributed equally.

https://doi.org/10.1016/j.plrev.2017.09.003

1571-0645/

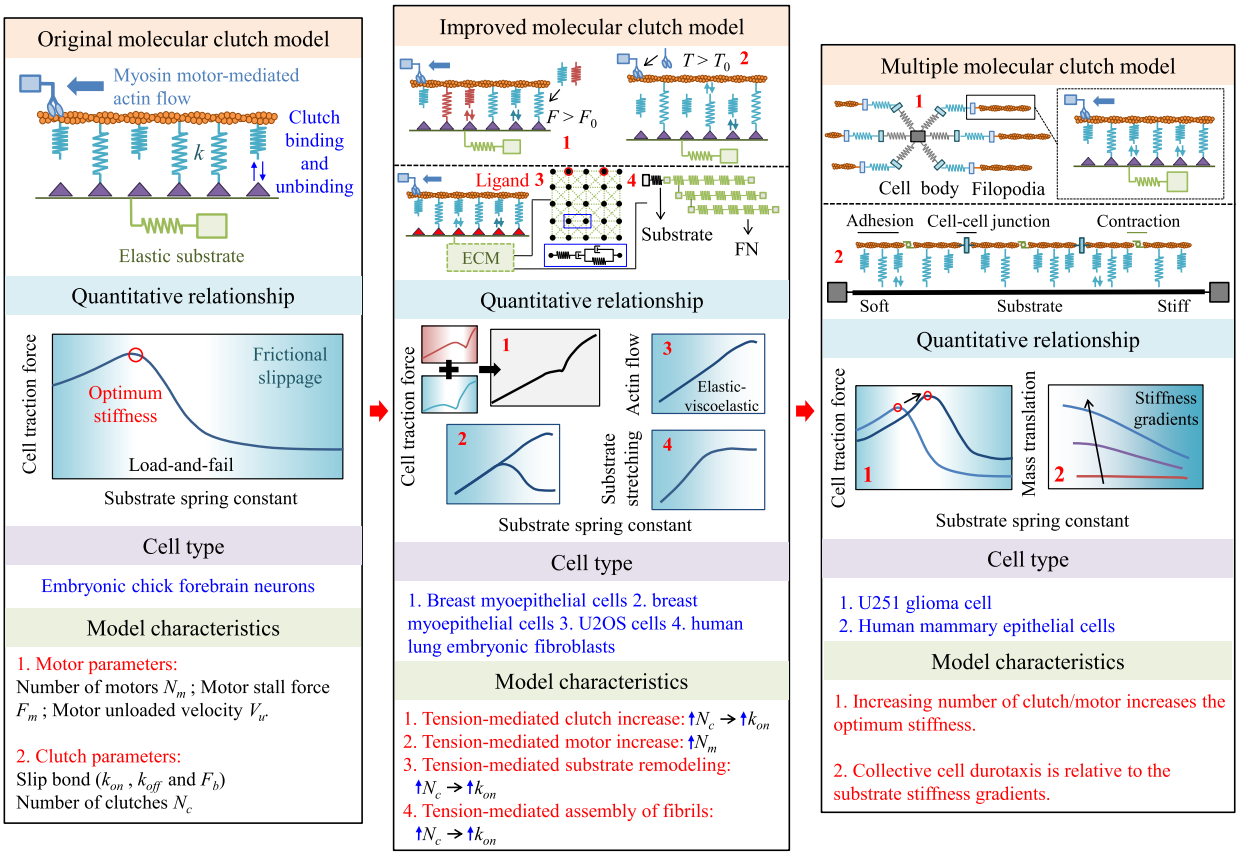

Fig. 1. The history of molecular clutch models.

Major advances in the field of mechanobiology have arisen from discussions of how complex behaviors might arise from a handful of simple principles, and we are honored to have the opportunity to reply to the three insightful commentaries of Nicolas [1], Spill et al. [2], and Wu et al. [3] that advance this discourse far beyond our short review [4]. These authors each noted that living cells exist outside of thermal equilibrium, and that the state-of-the-art models presented in our review must be called into question because they do not account for this.

1. Molecular clutch models and non-equilibrium steady states

A main focus of our review was molecular clutch models based upon the Mitchison and Kirschner [5] hypothesis that adhesion proteins are motor clutches that connect the actin cytoskeleton to the extracellular matrix (ECM), enabling transmission of forces from cells to ECM. We begin by reinforcing the observation that, despite their neglect of energetics, these models and their extensions provide valuable predictions.

The original molecular clutch model of Chan and Odde [6] models an elastic substrate loaded, through engaged molecular clutches, by myosin-mediated contraction and retrograde actin flow. Neither the model nor its inputs – motor and clutch parameters (**Fig. 1**) – account for energetics. However, predicted relationships between the cell traction and substrate stiffness match direct experimental observations, revealing a biphasic relationship consistent with the dynamics of single filopodial in embryonic chick forebrain neurons (ECFNs). However, this biphasic relationship is observed neither in EFCN growth cones, axons and neuronal soma, nor in 3T3 fibroblasts [7].

One possibility is certainly that an aspect of the non-equilibrium steady state character of living cells is not sustained outside of filopodial. The idea that energy supply to filopodial is enhanced by some kind of vesicle transport absent in these other parts of the neuron is certainly valid. As discussed in more detail in the final section of this commentary, such circular fluxes in non-equilibrium steady states are extraordinarily difficult to quantify. The ba-

Please cite this article in press as: Cheng B, et al. Energetics: An emerging frontier in cellular mechanosensing. Phys Life Rev (2017), https://doi.org/10.1016/j.plrev.2017.09.003

sic problem is summarized by Wyman [8], who likens energetics within a cell to those of a person on a treadmill: although reactions occur and energy is absorbed, the temperature, heat fluxes and concentrations of reactants and reaction byproducts remain unchanged at their steady state values. Without knowing that an irreversible process is underway, the steady state is nearly impossible to distinguish from, as Wyman calls it, "true equilibrium."

Before addressing this, we note that refined quasi-equilibrium models that neglect energetics continue to be highly successful, and continue to be refined with much success.

2. Improved molecular clutch models operating in an overwhelming supply of nutrients

Beginning by ignoring energetics, we note that refinements to quasi-static molecular clutch models have gone a long way towards resolving new observations as they emerge. From this perspective, why might the original Chan-Odde model not capture behaviors of EFCN growth cones, axons and neuronal soma, and 3T3 fibroblasts?

The first possibility that has been explored is whether model parameters are simply distinct in different types of cells. Odde and co-workers investigated the effects of clutch-motor parameters on cell traction force and actin flow [9] and found that parameter choice affected the substrate stiffness at which cell traction is maximized. Up-regulating clutch parameters increase this optimum stiffness, while up-regulating motor parameters decrease it, perhaps explaining why different types of cells display distinct force-stiffness relationships.

A second possibility is that focal adhesions connecting the cytoskeleton to the ECM might not be well modeled by the parallel springs of the molecular clutch model in other types of cells or different parts of EFCNs. For example, mechanical links between the actin cytoskeleton and substrate in the growth cone perhaps might be better modeled as an elastic network structure [10]. Fibroblasts have more stable focal adhesions and a higher optimal stiffness than neurons.

One-dimensional molecular clutch models cannot model whole cell behaviors such as cell spreading area and migration rate, but a two-dimensional extension of Bengasser et al. [11], with each protrusion at the edge of a cell modeled as a radial motor-clutch module, enables prediction of the cell body displacement by balancing forces. This model predicts that a decrease of motor-clutch number will decrease the stiffness that maximizes cell traction, in agreement with experiments on U251 glioma cells with chemically inhibited myosin motors and integrin-mediated adhesions. Consistent with this, ECFNs have both lower optimal stiffness and lower levels of myosin motors and integrin-mediated adhesions.

The discrete molecular clutch of García-Aznar and co-workers [12] adds three important factors: (1) diffusion of adhesion complexes; (2) tracking of both actin/adhesion complex and substrate/adhesion complex interfaces; and (3) unfolding of adhesion complex according to a saw-tooth tension-extension curve based upon talin-stretching experiments. The model also considers the effects on the clutch dynamics of force fluctuations in adhesion proteins arising from protein unfolding/refolding. The discrete model recovers the biphasic force-stiffness relationship, in agreement with the original molecular clutch model, despite the distinct clutch dynamics used. Extension to three dimensions [13] enabled investigation of how cell-adhesion size increases with cell traction-induced ECM alignment and with fiber diameter, ligand density and ECM cross-linker stiffness.

The third possibility we will discuss is time-dependent effects such as substrate viscosity. A molecular clutch model of cell spreading on viscoelastic substrata proposed by Mooney and co-workers [14] concludes that cell spreading is enhanced on certain viscoelastic substrata, consistent with experiment (**Fig. 1**). Local substrate remodeling (*e.g.*, clustering of adhesion ligands), arising cell traction-dependent flow and plastic deformation of the substrate, can increase the effective binding rate.

A fourth possibility is ECM kinetics, as in the molecular clutch model including fibronectin (FN) fibril assembly proposed by Lemmon and co-workers [15]. This model predicts that FN fibril assembly enables a transition from a biphasic to a monotonic force-stiffness relationship; this is, cells could indirectly sense substrate stiffness through traction-dependent FN dynamics. FN molecules, modeled with strain-dependent stiffness according to the wormlike chain model (**Fig. 1**), can unfold at a critical tension and promote additional FN binding within a hexagonally packed array. Whereas the Chan-Odde model predicts frictional slippage and load-and-fail regimes of cell response, the FN fibril assembly predicts an additional regime in which cell traction (and substrate stretching) remains high even on stiff substrata: on stiff substrata, FN binding sites are exposed frequently, resulting in more FN assembly and thus more integrin binding sites. From this point of view, an increasing binding rate of clutches will also appear at high substrate

Please cite this article in press as: Cheng B, et al. Energetics: An emerging frontier in cellular mechanosensing. Phys Life Rev (2017), https://doi.org/10.1016/j.plrev.2017.09.003

stiffness because of FN assembly. This improved molecular clutch model offers another successful explanation why some types of cells have a larger force on stiffer substrates, and a monotonic force-stiffness relationship.

The steady state kinetics of clutches and motors are clearly important [16], and the energetics and rate constants must still be measured. Roca-Cusachs and co-workers investigated how distinct bond dynamics of different integrin types (widely-expressed $\alpha_5\beta_1$ and selectively-expressed $\alpha_v\beta_6$) affect force-stiffness relationships [17]: $\alpha_v\beta_6$ has higher effective binding and unbinding rates and affinity with fibronectin than $\alpha_5\beta_1$. By also adding strengthening of molecular clutches beyond a critical force threshold, they obtained a model with three regimes in its force-stiffness relationship: (1) a frictional slippage regime at low stiffness; (2) a load-and-fail regime at intermediate stiffness; and (3) a reinforcement regime at high stiffness. The first two regimes are consistent with predictions of the Chan-Odde model, and the last one enables force-induced integrin density increases to increase cell traction at high substrate stiffnesses (**Fig. 1**). Most importantly, an almost monotonic force-stiffness relationship appears when cells have two types of integrins to bind competitively with FN.

In the comment of Nicolas [1], a reasonable explanation of why and how cells have larger adhesions on stiffer substrate is proposed. The author concludes that stress fibers or adhesions are less deformed on softer substrate, which leads to a lower cellular tension according to Hill's law, then down-regulating adhesion cryptic site unveiling-mediated adhesion reinforcing. This conclusion can also explain why cells have high internal cellular tension on stiff substrate. Recently, Roca-Cusachs and co-workers also found that talin unfolding-mediated vinculin binding can also eliminate produce a monotonic relationship which further validates such cellular mechanosensing mechanism from both experiments and computation model [18]. In their model, the FN-integrin bonds in actin-talin-integrin-FN clutches tend to break when force in the clutch drops below a threshold value, while talin in the clutch tends to unfold when above another threshold force. Our group developed a model in which a switch between monotonic and biphasic force-stiffness relationships is modulated by a "weakest link" in the adhesion dynamics: the adhesion dynamics and hence the force-stiffness relationship can be dominated by breakage of either the bond between the actin cytoskeleton and membrane-bound integrin, or a bond between membrane-bound integrin and extracellular FN [19] (**Fig. 1**).

3. Measuring non-equilibrium steady state rate constants: a critical open challenge

We conclude by agreeing with the three commentators that energetics is quite likely an important direction for future modeling efforts. The ability of a cell to provide ATP to a focal adhesion site could very well explain differences between the various observations in the literature for molecular clutch that have been observed in different cell types. Additionally, the variations within a cell might be explained by differing rate constants in local non-equilibrium study states.

However, these hypotheses are difficult to test at present. Underlying this difficulty is a major challenge in all of cell biology, namely measuring non-equilibrium steady-state rate constants and reaction fluxes. Although general methods do not yet exist, some techniques on the horizon do appear promising. The work of Chen, Elson, and collaborators points to promise for florescence correlation spectroscopy and fluorescence cross-correlation spectroscopy techniques [20–22].

More broadly, the energetics and reaction fluxes needed to achieve steady state within a cell might vary strongly as a function of the local disposition of the cytoskeleton and metabolic state of the cell. We agree fully that this is an important direction for future inquiry.

4. How to build a multiscale chemo-mechanical model to describe the cellular mechanosensing

In the comment of Spill et al., an important role of multiscale dynamics in cellular mechanosensing is also highlighted. Namely, it is important to understand how to link cell-adhesion formation, maturation, down-stream signalling pathways and further corresponding cellular behaviors in a multiscale chemo-mechanical model. For example, it has been shown that high substrate stiffness can upregulate the phosphorylation of focal adhesion kinase on Y397 (FAKpY397) within cell-adhesions among many types of cells [23]. Interestingly, in the comment of Wu et al., the authors also highlight the importance of chemo-mechanical coupling which could successfully explain many cellular mechanosensitive questions such as, stiffness-YAP-mediated cellular differentiation and stiffness-central dorsal ruffles-mediated cellular morphological changes. However, why and how the down-stream signals (FAK, Rho and YAP) are related to the substrate stiffness is still unknown. In these existing models, there are two kinds of

Please cite this article in press as: Cheng B, et al. Energetics: An emerging frontier in cellular mechanosensing. Phys Life Rev (2017), https://doi.org/10.1016/j.plrev.2017.09.003

phenomenological equations to describe the relationship of substrate stiffness (mechanical force) and intracellular molecular (*e.g.*, FAK): 1) the quantitative relationship between the activation rate of FAK (or ROCK) and substrate stiffness is described by a second-order Hill function [24]; 2) the FAK (ROCK) activity is linearly related to the log of substrate stiffness [25,26]. However, these two relationships are mainly based on qualitative tendency in the experimental observations but not the physical-principle-based theoretical models. Thus, these relationships cannot describe the accurate cell behaviors, such as the level of adhesion dynamics. In addition, the important dynamics of adhesions and their functions of activation of bio-signals are still elusive. Recently, Sheetz et al. showed that cell-adhesions are loose aggregates containing tight clusters of integrins with a size of ~ 100 nm composed of ~ 20–50 integrin molecules [27]. More importantly, it has been recently shown that the phosphorylation of FAKY397 only occurs within integrin clusters [28]. Based on these experimental observations, a single integrin cluster probably acts as a platform where intracellular chemical signals (*e.g.*, FAK) are activated that is dependent on substrate stiffness. Thus, the adhesion-mediated cellular mechanosensing may include three processes: integrin cluster formation on the plasma membrane, stiffness-dependent disassembly of integrin cluster and FAKY397 phosphorylation within integrin clusters. However, the quantitative relationships between these processes are still unsolved. In a word, a multiscale model including the cell-adhesion dynamics and signaling pathway dynamics is still urgently-needed.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (11372243, 11522219, 11532009, 11402192), the Chinese Ministry of Education through a Changjiang Scholar award to GMG, the National Institutes of Health through grants U01EB016422 and R01HL109505, and the NSF Science and Technology Center for Engineering Mechanobiology, grant CMMI 1548571.

References

[1] Nicolas A. Cell adhesion mechanosensitivity, an active biological process. Comment on "Cellular mechanosensing of the biophysical microenvironment: A review of the mathematical models of biophysical regulation of cell responses" by Bo Cheng et al. Phys Life Rev 2017 [in this issue].
[2] Spill F, Zaman M. Multiscale dynamics of the biophysical and biochemical microenvironment. Comment on "Cellular mechanosensing of the biophysical microenvironment: A review of mathematical models of biophysical regulation of cell responses" By Bo Cheng et al. Phys Life Rev 2017. http://dx.doi.org/10.1016/j.plrev.2017.07.004 [in this issue].
[3] Wu J, Steward R Jr. How can we predict cellular mechanosensation? Comment on "Cellular mechanosensing of the biophysical microenvironment: A review of mathematical models of biophysical regulation of cell responses" by Bo Cheng et al. Phys Life Rev 2017. http://dx.doi.org/10.1016/j.plrev.2017.08.013 [in this issue].
[4] Cheng B, Lin M, Huang G, Li Y, Ji B, Genin GM, et al. Cellular mechanosensing of the biophysical microenvironment: A review of mathematical models of biophysical regulation of cell responses. Phys Life Rev 2017. http://dx.doi.org/10.1016/j.plrev.2017.06.016 [in this issue].
[5] Mitchison T, Kirschner M. Cytoskeletal dynamics and nerve growth. Neuron 1988;1:761–72.
[6] Chan CE, Odde DJ. Traction dynamics of filopodia on compliant substrates. Science 2008;322:1687.
[7] Han Sangyoon J, Bielawski Kevin S, Ting Lucas H, Rodriguez Marita L, Sniadecki Nathan J. Decoupling substrate stiffness, spread area, and micropost density: a close spatial relationship between traction forces and focal adhesions. Biophys J 2012;103:640–8.
[8] Jeffries W. The turning wheel: a study in steady states. Proc Natl Acad Sci USA 1975;72:3983.
[9] Bangasser B, Rosenfeld S, Odde D. Determinants of maximal force transmission in a motor-clutch model of cell traction in a compliant microenvironment. Biophys J 2013;105:581–92.
[10] Mejean CO, Schaefer AW, Buck KB, Holger K, Alla S, Merrill JW, et al. Elastic coupling of nascent apCAM adhesions to flowing actin networks. PLoS ONE 2013;8:e73389.
[11] Bangasser BL, Shamsan GA, Chan CE, Opoku KN, Tüzel E, Schlichtmann BW, et al. Shifting the optimal stiffness for cell migration. Nat Commun 2017;8:15313.
[12] Escribano J, Sánchez MT, García-Aznar JM. A discrete approach for modeling cell-matrix adhesions. Comput Part Mech 2014;1:117–30.
[13] Escribano J, Sánchez MT, García-Aznar JM. Modeling the formation of cell-matrix adhesions on a single 3D matrix fiber. J Theor Biol 2015;384:84–94.
[14] Chaudhuri O, Luo G, Darnell M, Klumpers D, Bencherif SA, Weaver JC, et al. Substrate stress relaxation regulates cell spreading. Nat Commun 2015;6:6364.
[15] Weinberg SH, Mair DB, Lemmon CA. Mechanotransduction dynamics at the cell-matrix interface. Biophys J 2017;112:1962–74.
[16] Bangasser BL, Odde DJ. Master equation-based analysis of a motor-clutch model for cell traction force. Cell Mol Bioeng 2013;6:449–59.
[17] Elosegui-Artola A, Bazellières E, Allen M, Andreu I, Oria R, Sunyer R, et al. Rigidity sensing and adaptation through regulation of integrin types. Nat Mater 2014;13:631–7.
[18] Elosegui-Artola A, Oria R, Chen Y, Kosmalska A, Pérez-González C, Castro N, et al. Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. Nat Cell Biol 2016;18:540–51.

[19] Cheng B, Lin M, Li Y, Huang G, Yang H, Genin GM, et al. An integrated stochastic model of matrix-stiffness-dependent filopodial dynamics. Biophys J 2016;111:2051–61.
[20] Qian H, Elson EL. Single-molecule enzymology: stochastic Michaelis–Menten kinetics. Biophys Chem 2002;102:565–76.
[21] Qian H, Elson EL. Fluorescence correlation spectroscopy with high-order and dual-color correlation to probe nonequilibrium steady states. Proc Natl Acad Sci USA 2004;101:2828–33.
[22] Qian H, Saffarian S, Elson EL. Concentration fluctuations in a mesoscopic oscillating chemical reaction system. Proc Natl Acad Sci USA 2002;99:10376–81.
[23] Wei WC, Lin HH, Shen MR, Tang MJ. Mechanosensing machinery for cells under low substratum rigidity. Am J Physiol, Cell Physiol 2008;295:C1579.
[24] Sun M, Spill F, Zaman M. A computational model of YAP/TAZ mechanosensing. Biophys J 2016;110:2540–50.
[25] Schroer AK, Ryzhova LM, Network Merryman WD. Modeling approach to predict myofibroblast differentiation. Cell Mol Bioeng 2014;7:446–59.
[26] Zeng Y, Lai T, Koh CG, Leduc P, Chiam KH. Investigating circular dorsal ruffles through varying substrate stiffness and mathematical modeling. Biophys J 2011;101:2122–30.
[27] Changede R, Xu X, Margadant F, Sheetz MP. Nascent integrin adhesions form on all matrix rigidities after integrin activation. Dev Cell 2015;35:614–21.
[28] Seong J, Tajik A, Sun J, Guan J, Humphries MJ, Craig SE, et al. Distinct biophysical mechanisms of focal adhesion kinase mechanoactivation by different extracellular matrix proteins. Proc Natl Acad Sci USA 2013;110:19372.

Please cite this article in press as: Cheng B, et al. Energetics: An emerging frontier in cellular mechanosensing. Phys Life Rev (2017), https://doi.org/10.1016/j.plrev.2017.09.003