Calibrating Kinetic Rate Constants of Signaling Cascades Involved in Cell Mechanotransduction at Junctions

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Cells have different modes of communication. Biochemical cues have been quite well studied in the past while biophysical cues are novel and unchartered territory. The non-canonical hippo pathway is one such pathway that responds to changes in mechanical properties and cues like tension. This study focuses on simulating how junction proteins specifically E-cadherin and focal adhesions transduce forces and transmits this signal downstream; In Focal Adhesions, activated Talin and Vinculin recruits Focal Adhesion Kinase which activates Rac1, cdc42 and Rho proteins that polymerizes the actin cytoskeleton. In Cadherin junctions, stretching of the proteins recruits vinculin which remodels the actin cytoskeleton. The signaling cascades have been assembled on Simulink, MATLAB and sample force-extension data from other experiments have been used to perform a parameter estimation to calculate the rate constants in each of the reactions involved to demonstrate a proof of concept of the method.

Introduction

Mechanotransduction is the phenomenon by which a protein via conformational or other physical or chemical modifications due to mechanical signals transmit information (*1*). For example, the protein Talin, when subjected to tensile forces, stretch and expose vinculin binding sites which further conveys that signal via downstream protein-protein interactions (*2*). For example, application of tensile loads on cells causes polymerization of G-actin to F-actin to cater to that load, which may influence the concentration of specific proteins like YAP/TAZ (*3*). Hence, modeling the response of the cell to such mechanical signals via cytoskeletal remodeling could increase the understanding of how phenomena dependent on mechanical inputs such as contact inhibition occurs.

The study presented here proposes a method whereby, experimental rheology data of single cells can be used to kinetically calibrate it. Knowing the kinetic rate constants can then provide valuable insight into how the afore-mentioned signaling cascades transmit information.

The common methods of performing the analysis is to find the reaction rate constants for each protein interaction and then simulate them. However, this is quite difficult and prolongs the study excessively. For systems such as the one studied here, that have both input and output data, this method would simplify the problem by making use of advanced optimization techniques.

Methods

With the advent of experimental tools like Optical Traps and Atomic Force Microscopy, it has become possible to obtain stress-strain hysteresis loops of single cells (*4*). These are stress vs strain loops that gives valuable information about how the cell responds to an applied force and how quickly. The hysteresis loops of a material describes both the stiffness and the damping constant for the material under loads applied at a set frequency.

The actin cytoskeleton redistributes the stress experienced by the cell (*5*). Hence, as stresses increase, the cytoskeleton must remodel or re-arrange itself - or make the cell more stiff in response. Assuming that the stiffness of a cell

is directly proportional to the concentration of polymerized F-actin, different points on the hysteresis loop correspond to different levels of F-actin concentration.

Next, the internal mechanism of the cell that produces this change was mapped. The cell contains several junction proteins such as tight junctions, adherens junction, focal adhesions, desmosomes and hemidesmosomes. Among these the adherens junction and the focal adhesion are connected to the actin cytoskeleton via the proteins E-cadherin and $\alpha\beta$ integrin (*6*). Due to their connection to the actin network, they have been proven to play important roles in transmitting mechanical signals that remodel the network (*5, 7–9*).

The signaling cascades that model these interactions were obtained from literature.

Focal Adhesion Pathway

The Focal Adhesion complex contains a large family of up to 150 different kinds of proteins. Integrins present on the plasma membrane have an extra-cellular and intra-cellular domain. Mechanosensitive Talin protein bind to the intracellular region of integrin. Once, Talin is activated by tension, vinculin binds to it (*10*). The Talin-Vinculin complex is coupled to the retrograde flow of actin filaments. This activation also recruits the Focal Adhesion Kinase (FAK), which upon phosphorylation activates Rho - in turn activating mDIA and ROCK proteins which catalyzes the polymerization of G-actin (*11*). The formation of FAK-Src complex also recruits proteins like paxillin and p130Cas (*12*) which in the presence of Crk recruits βPix and DOCK180 (*13*) which also catalyzes the polymerization via Rac1 (*14*) and CDC42 proteins respectively (*15*).

E-cadherin Pathway

E-cadherin is the junction protein present at the adherens junction. It is responsible for keeping adjacent cells in contact. In the unperturbed state, E-cadherin is connected to α catenin, which along with vinculin are bound to f-actin stress fibers. When E-cadherin experiences a tensile force, vinculin is activated and recruits formin and other proteins that result in cytoskeletal remodeling (*16*).

Modeling

In this model, the interactions of the junction proteins with its effectors are assumed to be localized to the junctions. The spatial variations of the proteins are not considered. This enables modeling the system as ordinary differential equations. Each equation is characterized by a forward and reverse kinetic rate constant. In total, the model uses 52 such rate constants.

The initial concentration of all the involved proteins were assumed to be 0.01 M and all the rate constants had an initial guess of 1.

All the reactions were modeled using simple Michaelis-Menten kinetics for Receptor-Ligand binding for activation of proteins and for the catalysis reactions.

The ordinary differential equations governing these interactions were modeled as Simulink block diagrams with a force input and the f-actin concentration as the output.

Parameter Estimation

In order to perform parameter estimation, the Simulink block diagram requires displacement (input) and force (output) time signals. For the purpose of this study, an artificially generated (not experimental data) hysteresis loop was used. The data was scaled down to the characteristic order of magnitude of the cell e.g: nN-vs-μm.

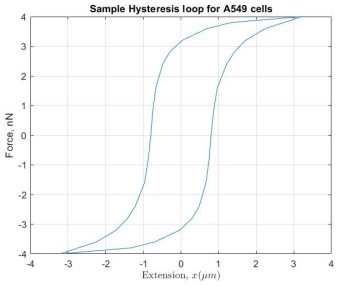

Fig. 1 An example hysteresis loop

Using these hysteresis loops, the dynamic stiffness of the cell was calculated for each value of the force using a simple forward difference scheme. This is the output signal to be used by the model. The input displacement signal was a sinusoidal signal of amplitude taken from the maximum displacement on the hysteresis loop and a frequency of 10 Hz. This data was used in the Simulink block diagram and specified in the parameter estimation dialogue box of the design optimization tool.

A sum of least squares algorithm was used to optimize the system or to tune the kinetic rate constants involved in the block diagrams.

Results

Using the initial conditions of the protein concentrations and the ki-

netic rate constants, the parameter estimation process was executed. The program was able to tune some of the rate constants and minimized the error from 8000 to 2000. See the results of the parameter estimation in the supplemental section.

Discussion

The rate constants being optimized will approach the actual rate constants of protein interactions when the sum of squares of errors is reduced to a small value - preferably of the order of 10^{-7} at least.

Knowing the rate constant for each of the interactions, the Simulink block diagram provides a transfer function between the force and the f-actin concentration. F-actin directly causes the nuclear translocation of the YAP and TAZ proteins. Hence, the model offers a simple way to relate the applied force and the translocation of the proteins. Using this model and experiments, the exact amount of force that initiates the cell's decision to engage in contact inhibition can be estimated.

One way of testing the model would be to apply forces to cells using an optical trap and measuring the translocation of the YAP/TAZ proteins.

Apart from designing the transfer function, the model predicts to some accuracy the expected rate constants and may help design experiments for determining these rate constants or numerically validating them for an in-vivo case if they are already known.

In order to improve the model's ability to predict the rate constant, the algorithm employed and the data used needs to be improved. A model with such a large number of parameters will have multiple local minima inside the cost-parameter space. Hence, a more intelligent algorithm needs to be developed to provide better results. Also, the data used for estimation was of single amplitude and did not characterize multiple amplitudes or frequency and was not realistic in that regard. Another idea is to perform a Principle Component Analysis. This will filter out some of the parameters that do not exert as much influence as others (different in orders of magnitude)

The study conducted here investigates only a small portion of a large signaling cascade. Also, it does not consider the possible crosstalk between the focal adhesion and adherens junction mechano-sensors. This is common for many signaling cascades and will be addressed in future studies.

However, despite the many issues, the study offers a proof-of-concept for a modeling practice that can enable researchers to extract chemical kinetic rate data from mechanical data in cells and use it to obtain physiologically relevant information via transfer functions of this nature.

References and Notes

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