The Role of Notch Signaling in Mechanical-Tension Regulation of H2-Calponin Gene

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The essential role of mechanical signaling in regulating the cellular function of living organisms has been widely recognized. However, how mechanical signals are transduced in cells to regulate gene expression and other biochemical activities is not well understood. Our previous studies have demonstrated that the gene encoding h2-calponin (Cnn2) is regulated by mechanical tension (Hossain et al., JBC 280:42442-53, 2005). The data indicated that cis-regulatory element(s) located between –1.6-kb and –1.4-kb upstream of the mouse Cnn2 gene is responsible for the mechanical tension-regulation. The present study quantitatively studied the regulation of h2-calponin gene by mechanical tension in the cytoskeleton focusing on this region. Potential transcriptional regulatory factor binding sites within the –1.6 to –1.4-kb region were explored using reporter gene constructs in cells cultured on high and low stiffness substrates or in comparison between floating and adherent cultures. The results indicated a role of HES1 downstream of the Notch signaling pathway. While floating cultures of C2C12 myoblasts showed a significant decrease in h2-calponin expression compared to the adherent cultures, treatments with a Notch pathway inhibitor DAPT were able to minimize these effects. Our findings suggest that global properties of RNA dynamics require a reassessment of our understanding of cellular heterogeneity and stochastic gene expression, and further suggest that evolution selects for particular genes to have certain transcriptional parameters in order to maintain their density. Our results suggest that density conservation is a natural consequence of the global feedback between cell volume and mRNA abundance independent of any specific mechanism.

IFN-Independent Expression of RIG-I is a Determinant of Heterogeneous IFN-B Expression States in Antiviral Signaling

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RIG-I plays a major role in antiviral innate immunity by detecting the cytoplasmic viral RNA and triggering the pathway that leads to transcriptional activation of type I interferon (IFN). Type I IFN induces a large set of genes called interferon stimulated genes (ISGs) which coordinate to antagonize viral evasion. We utilized single-molecule fluorescence in situ hybridization (smFISH) to study viral and antiviral gene expression in individual cells. We precisely quantified kinetics of mRNA expression of RIG-I, IFN-b and Sendai virus (SeV) L gene in clonal mammalian cells upon infection with SeV. Contrary to the expectation that RIG-I expression is only up-regulated through positive feedback involving Type I IFN, we found that RIG-I is directly induced by viral infection in the absence of IFN at the early stages of viral infection. Examination of expression kinetics of a subset of ISGs showed the differential regulation of ISGs in response viral infection. MDA5, LGP2, OasL and Viperin were directly activated by SeV infection before IFN-b. On the other hand, activation of some other ISGs was found to be dependent on IFN signaling, such as PKR, MxA, IRF7, TRIM25, and NLRX1. smFISH experiments revealed two distinct populations of cells which would otherwise be obscured in ensemble measurements: IFN-b expressing and IFN-b non-expressing cells. Simultaneous counting of IFN-b, RIG-I and SeV L gene mRNA and correlation analysis in individual cells revealed that transcriptional activation of IFN-b is dependent on the level of RIG-I mRNA in individual cells that is IFN-independently induced at the early stages of viral infection but is independent of the level of viral replication.

Predicting Rates of Cell State Change due to Stochastic Fluctuations using a Data-Driven Landscape Model

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We develop a potential landscape approach to quantitatively describe experimental data from a fibroblast cell line that exhibits a wide range of green fluorescent protein (GFP) expression levels under the control of the promoter for tenascin-C. Time lapse live cell microscopy provides data about short term fluctuations in promoter activity, and flow cytometry measurements provide data about the long term kinetics as isolated subpopulations of cells relax from a relatively narrow distribution of GFP expression back to the original broad distribution of responses. The landscape is obtained from the steady state distribution of GFP expression and is connected to a potential-like function using a stochastic differential equation description (Langevin/Fokker-Planck). The range of cell states is constrained by a “force” that is proportional to the gradient of the potential, and biochemical noise causes the movement of cells within the landscape. Analyzing the mean square displacement of GFP intensity changes in live cells indicates that these fluctuations are described by a single diffusion constant in log GFP space. This allows application of the Kramers’ model to calculate rates of switching between two attractor states, and enables an accurate simulation of the dynamics of relaxation back to the steady state with no adjustable parameters. With this approach it is possible to use the steady state distribution of phenotypes and a quantitative description of the short term fluctuations in individual cells to predict the rates at which different phenotypes will arise from an isolated subpopulation of cells.