Year of publication 2019

Stankevicins L, Ecker N, Terriac E, Maiuri P, Schoppmeyer R, Vargas P, Lennon-Duménil AM, Piel M, Qu B, Hoth M, Kruse K, Lautenschläger F. (2020 Jan 14)

Deterministic actin waves as generators of cell polarization cues.

Proceedings of the National Academy of Sciences : 117 : Proc Natl Acad Sci U S A. 2020 Jan 14;117(2):826-835. doi: 10.1073/pnas.1907845117. Epub 2019 Dec 27. : 826,835 : DOI : 10.1073/pnas.1907845117

Summary

Dendritic cells “patrol” the human body to detect pathogens. In their search, dendritic cells perform a random walk by amoeboid migration. The efficiency of pathogen detection depends on the properties of the random walk. It is not known how the dendritic cells control these properties. Here, we quantify dendritic cell migration under well-defined 2-dimensional confinement and in a 3-dimensional collagen matrix through recording their long-term trajectories. We find 2 different migration states: persistent migration, during which the dendritic cells move along curved paths, and diffusive migration, which is characterized by successive sharp turns. These states exhibit differences in the actin distributions. Our theoretical and experimental analyses indicate that this kind of motion can be generated by spontaneous actin polymerization waves that contribute to dendritic cell polarization and migration. The relative distributions of persistent and diffusive migration can be changed by modification of the molecular actin filament nucleation and assembly rates. Thus, dendritic cells can control their migration patterns and adapt to specific environments. Our study offers an additional perspective on how dendritic cells tune their searches for pathogens.

Sáez JJ1,2, Diaz J1, Ibañez J1, Bozo JP1, Cabrera Reyes F1, Alamo M1, Gobert FX3, Obino D3, Bono MR2, Lennon-Duménil AM3, Yeaman C4, Yuseff MI5. (2019 Jul 1)

The exocyst controls lysosome secretion and antigen extraction at the immune synapse of B cells.

Journal of cell biology : 218(7) : :2247-2264 : DOI : 10.1083/jcb.201811131

Summary

Year of publication 2016

A Coulon, D R Larson (2016 Jun 1)

Fluctuation Analysis: Dissecting Transcriptional Kinetics with Signal Theory.

Methods in enzymology : 159-91 : DOI : 10.1016/bs.mie.2016.03.017

Summary

Recent live-cell microscopy techniques now allow the visualization in multiple colors of RNAs

as they are transcribed on genes of interest. Following the number of nascent RNAs over time at a single locus reveals complex fluctuations originating from the underlying transcriptional kinetics. We present here a technique based on concepts from signal theory-called fluctuation analysis-to analyze and interpret multicolor transcriptional time traces and extract the temporal signatures of the underlying mechanisms. The principle is to generate, from the time traces, a set of functions called correlation functions. We explain how to compute these functions practically from a set of experimental traces and how to interpret them through different theoretical and computational means. We also present the major difficulties and pitfalls one might encounter with this technique. This approach is capable of extracting mechanistic information hidden in transcriptional fluctuations at multiple timescales and has broad applications for understanding transcriptional kinetics.

Year of publication 2015

Tineke L Lenstra, Antoine Coulon, Carson C Chow, Daniel R Larson (2015 Nov 10)

Single-Molecule Imaging Reveals a Switch between Spurious and Functional ncRNA Transcription.

Molecular cell : 597-610 : DOI : 10.1016/j.molcel.2015.09.028

Summary

Eukaryotic transcription is pervasive, and many of the resulting RNAs are non-coding. It is unknown whether ubiquitous transcription is functional or simply reflects stochastic transcriptional noise. By single-molecule visualization of the dynamic interplay between coding and non-coding transcription at the GAL locus in living yeast cells, we show that antisense GAL10 ncRNA transcription can switch between functional and spurious under different conditions. During galactose induction, GAL10 sense transcription occurs in short stochastic bursts, which are unaffected by transcription of antisense GAL10 ncRNA, even when both are present simultaneously at the same locus. In contrast, when GAL10 is not induced, ncRNA transcription is critical to prevent transcriptional leakage of GAL1 and GAL10. Suppression of ncRNA transcription by strand-specific CRISPR/dCas9 results in transcriptional leakage of the inducer GAL1, leading to a more sensitive transcription activation threshold, an alteration of metabolic switching, and a fitness defect in competition experiments.

Diana A Stavreva, Antoine Coulon, Songjoon Baek, Myong-Hee Sung, Sam John, Lenka Stixova, Martina Tesikova, Ofir Hakim, Tina Miranda, Mary Hawkins, John A Stamatoyannopoulos, Carson C Chow, Gordon L Hager (2015 Feb 14)

Dynamics of chromatin accessibility and long-range interactions in response to glucocorticoid pulsing.

Genome research : 845-57 : DOI : 10.1101/gr.184168.114

Summary

Although physiological steroid levels are often pulsatile (ultradian), the genomic effects of this pulsatility are poorly understood. By utilizing glucocorticoid receptor (GR) signaling as a model system, we uncovered striking spatiotemporal relationships between receptor loading, lifetimes of the DNase I hypersensitivity sites (DHSs), long-range interactions, and gene regulation. We found that hormone-induced DHSs were enriched within ± 50 kb of GR-responsive genes and displayed a broad spectrum of lifetimes upon hormone withdrawal. These lifetimes dictate the strength of the DHS interactions with gene targets and contribute to gene regulation from a distance. Our results demonstrate that pulsatile and constant hormone stimulations induce unique, treatment-specific patterns of gene and regulatory element activation. These modes of activation have implications for corticosteroid function in vivo and for steroid therapies in various clinical settings.

Year of publication 2014

Antoine Coulon, Matthew L Ferguson, Valeria de Turris, Murali Palangat, Carson C Chow, Daniel R Larson (2014 Oct 2)

Kinetic competition during the transcription cycle results in stochastic RNA processing.

eLife : DOI : 10.7554/eLife.03939

Summary

Synthesis of mRNA in eukaryotes involves the coordinated action of many enzymatic processes, including initiation, elongation, splicing, and cleavage. Kinetic competition between these processes has been proposed to determine RNA fate, yet such coupling has never been observed in vivo on single transcripts. In this study, we use dual-color single-molecule RNA imaging in living human cells to construct a complete kinetic profile of transcription and splicing of the β-globin gene. We find that kinetic competition results in multiple competing pathways for pre-mRNA splicing. Splicing of the terminal intron occurs stochastically both before and after transcript release, indicating there is not a strict quality control checkpoint. The majority of pre-mRNAs are spliced after release, while diffusing away from the site of transcription. A single missense point mutation (S34F) in the essential splicing factor U2AF1 which occurs in human cancers perturbs this kinetic balance and defers splicing to occur entirely post-release.

Year of publication 2013

Antoine Coulon, Carson C Chow, Robert H Singer, Daniel R Larson (2013 Jul 10)

Eukaryotic transcriptional dynamics: from single molecules to cell populations.

Nature reviews. Genetics : 572-84 : DOI : 10.1038/nrg3484

Summary

Transcriptional regulation is achieved through combinatorial interactions between regulatory

elements in the human genome and a vast range of factors that modulate the recruitment and activity of RNA polymerase. Experimental approaches for studying transcription in vivo now extend from single-molecule techniques to genome-wide measurements. Parallel to these developments is the need for testable quantitative and predictive models for understanding gene regulation. These conceptual models must also provide insight into the dynamics of transcription and the variability that is observed at the single-cell level. In this Review, we discuss recent results on transcriptional regulation and also the models those results engender. We show how a non-equilibrium description informs our view of transcription by explicitly considering time- and energy-dependence at the molecular level.