Deuxième réunion annuelle du GDR Architecture et Dynamique du Noyau et des Génomes (ADN&G)

Rapport sur les contributions

https://indico.in2p3.fr/e/26886

ID de Contribution: **43** Type: **Non spécifié**

3DGenBench: A web-server to benchmark computational models for 3D Genomics

Modeling 3D genome organisation has been booming in the last years thanks to the availability of experimental datasets of genomic contacts. However, the field is currently missing the standardisation of methods and metrics to compare predictions and experiments. We present 3DGenBench, a web server available at https://inc-cost.eu/benchmarking/, that allows benchmarking computational models of 3D Genomics. The benchmark is performed using a manually curated dataset of 39 capture Hi-C profiles in wild type and genome-edited mouse cells, and five genome-wide Hi-C profiles in human, mouse, and Drosophila cells. 3DGenBench performs two kinds of analysis, each supplied with a specific scoring module that compares predictions of a computational method to experimental data using several metrics. Using 3DGenBench, the user obtains model performance scores, allowing an unbiased comparison with other models. 3DGenBench aims to become a reference web server to test new 3D genomics models and is conceived as an evolving platform where new types of analysis will be implemented in the future.

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ID de Contribution: **44** Type: **Non spécifié**

Characterizing the 3D organization of holocentric chromosomes in Bombyx mori

The centromere's primary role is to ensure chromosome segregation by catalyzing the formation of the kinetochore complex to promote attachment between spindle fibers and chromosomes during cellular division. In addition to this well-known function, centromeres are classic examples of chromosome regions known to impact the regulation and spatial architecture of the surrounding genome (Muller, Gil Jr and Drinnenberg, 2019). Microscopy studies and Chromosome Conformation Capture experiments (3C, Hi-C) have shown that centromeres cluster together in the nucleus of some organisms and they also act as strong topological barriers preventing specific types of contacts between the two chromosomal arms. These observations have mostly been made by studying monocentric organisms, or organisms that have chromosomes with a single centromere. Very little is known about the effect of centromeres on genome architecture in holocentric organisms, or organisms that have centromeres distributed along the entire length of their chromosomes. Still, a detailed characterization of this interplay using next generation sequencing approaches has been hindered in most model organisms, including humans, due to the repetitive nature of their centromeres. Our data revealing a non-repetitive nature of *Bombyx mori* centromeres offers a unique opportunity to circumvent the challenges usually presented when studying centromeres.

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ID de Contribution: **45** Type: **Non spécifié**

Role of spatial chromatin organization in recombinational DNA repair and genomic stability

Abstract

Homologous recombination (HR) templates DNA double-strand break (DSB) repair off an intact homologous dsDNA "donor"molecule, which can exist in the form of a sister chromatid, a homologous chromosome, or dispersed repeats. HR fidelity partly depends on this competitive donor selection process, which embeds homology sampling by the RecA/Rad51-ssDNA nucleoprotein filament (NPF), DNA joint molecules reversal by ancillary HR factors and, presumably, regulation of the spatial collisions between the NPF and any given genomic loci (Savocco and Piazza, 2021). We developed various proximity ligation-based methodologies enabling detection of transient NPF-dsDNA interactions and early DNA joint molecules (as-of-yet recalcitrant to molecular detection) which granted direct study of these core HR steps and their regulation in S. cerevisiae (Piazza et al., 2017, 2018, 2019, 2021a). In collaboration with the Koszul lab, we recently identified two main ways by which cohesin bias ectopic donor identification, promoting it in cis and inhibiting it in trans (Piazza et al., 2021b). We present here additional mechanistic insights into the promotion of cis-sampling by the NPF. Mutation accumulation experiments in genotoxic conditions reveals that perturbation of chromatin loop folding in hypomorph mutants of cohesin regulators leads to repeat-mediated genome instability. This preliminary work furthers the characterization of the cohesin-mediated regulation of homology search during HR and its role as a suppressor of genomic instability.

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Savocco, J., and Piazza, A. (2021). Recombination-mediated genome rearrangements. Curr. Opin. Genet. Dev. 71, 63–71.

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ID de Contribution: **46** Type: **Non spécifié**

Bayesian inference on a 3D polymer model for epigenetic data

The spatial conformation of chromatin within the nucleus is of the uttermost importance for transcriptional regulation and therefore for phenomena such as cell differentiation. In particular, epigenetic modifications play a crucial role as they are believed to affect the spatial folding and the accessibility of the genome to transcription factors and proteins.
Understanding and quantifying the interplay between the intrinsic polymeric nature of the chromatin fiber and its 3d folding in space that we have on one side and the 1d DNA sequence and its epigenetic content on the other remains an open challenge.
Here, we propose a magnetic polymer model with inhomogeneous, genomic-sequence dependent, external fields to describe said epigenetic modifications. Furthermore, we introduce a novel numerical method to perform approximate posterior inference on the free parameters of the model.

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ID de Contribution: **47** Type: **Non spécifié**

Recombination-transcription conflicts: from DNA joint molecule metabolism to repair outcome

Homologous recombination is a template-dependent DNA double-strand break repair mechanism. A key step of HR is DNA strand invasion, which consists of the pairing of the single-stranded DNA flanking the break site to a complementary strand in a homologous double-stranded DNA (dsDNA) leading to the formation of a DNA joint molecule called a D-loop. D-loop metabolism is likely central in the donor selection process, regulated by various conserved trans-acting factors involved in genome maintenance, such as the Mph1FANCM and Srs2FBH1 helicases, as well as the Sgs1-Top3-Rmi1BLM-TOPO3-RMI1/2 complex. However, little is known about the role of transcription, a ubiquitous and presumably competing cis-acting DNA-dependent event, on the core steps of homologous recombination. The goal of my project is to investigate the potential conflicts between recombination and transcription and their consequences for genome stability in S. cerevisiae. To this end, we used an assay for D-loop quantification and initiation of DNA synthesis at an ectopic donor site whose transcription can be modulated at will. We show that colinear transcription is the main cause of nascent D-loop disruption, and acts independently of the aforementioned trans disruption activities. This effect is acute, as inducing donor transcription for a few minutes prior to sample collection recapitulates D-loop inhibition by strong constitutive promoters. Moreover, it inhibits the initiation of DNA synthesis. Finally, transcription lowers the frequency of multi-invasions-induced rearrangements. These preliminary results suggest that the act of transcription represents a potent anti-recombination mechanism unevenly protecting the genome against rearrangements.

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ID de Contribution: **48** Type: **Non spécifié**

Genome stability: Interplay between DSB, chromatin mobility and SMC complexes

The genome is susceptible to multiple damages, including DNA double-strand breaks (DSBs) and replication stress. Chromosome configuration of the Saccharomyces cerevisiae plays a critical role in maintaining genome integrity upon damage, as only DSBs near pericentromeres cause an increase in global mobility, protective for the genome (1). Here, we want to understand what makes the pericentromere a special region. An interesting candidate is the conserved Smc5/6 complex that is part of the SMCs (structural maintenance complexes). This complex is enriched at centromeres, is recruited to DSBs and in yeast interacts with both microtubules and DNA (2,3). The interaction with microtubules is mediated by three lysines in the Smc5 protein (K624, K631 and K667). We hypothesized that mutations in these residues would impact chromatin mobility around the pericentromere. We chose to mutate the two lysines (K624 and K631), present in the characteristic hinge of Smc5. These mutations lead to increased chromatin mobility near the pericentromere, even in the absence of damage, in a microtubule-dependent manner. Microtubules are involved in genome mobility as well as maintaining the tension between kinetochores, which is essential for the error-free chromosome segregation. Interestingly, we show that the distances between spindle pole bodies in dividing cells, but not the distance between the spindle pole and the centromere, increase in the mutant. Moreover, the mutant presents a declustering of centromeres. These last observations suggest that mutations may cause a tension defect. Altogether, our results suggest a possible role of Smc5/6 in maintaining the tension when chromosomes segregate that could be correlated with the increase in chromosome mobility.

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ID de Contribution: **49** Type: **Non spécifié**

Interplays between nucleosomes, the histone chaperone FACT and condensin shape mitotic chromosomes

A conserved, yet incompletely understood, principle in living organisms is the folding of the genome into loops by DNA-translocases of the SMC family1. Upon mitotic entry, the condensin SMC complex binds DNA and shapes metaphase chromosomes by folding chromatin into loops, in preparation for genome segregation in anaphase. How condensin achieves this task remains unclear. The active extrusion of naked DNA into loops of increasing size by condensin observed in vitro convincingly describes the structural properties of mitotic chromosomes. Yet, the consubstantial issue of roadblocks and steric hindrances raises the salient question as to whether and how condensin could conceivably extrude loops in the context of a chromatinized genome. Conflicting results have been obtained thus far regarding the impact of nucleosomes on condensin, leaving the question unanswered. Using Fission yeast as a model system, we obtained evidence that arrays of nucleosomes hinder condensin in vivo, and that the histone chaperone FACT, known for its role in modulating nucleosome structure, associates with condensin and takes part in the formation of mitotic chromosomes. The role(s) played by FACT as an ancillary factor for condensin in the context of chromatin will be discussed.

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ID de Contribution: **50** Type: **Non spécifié**

Evidence that Rrp6/EXOSC10 is involved in condensin-mediated chromosome segregation in fission yeast

RNAs and transcription are emerging as important players in chromosome assembly and dynamics. Although the idea of a rigid and stable RNA scaffold has now been abandoned, several lines of evidence suggest that RNA form a dynamic mesh that controls chromatin structure. Moreover, transcription has been identified as a roadblock for SMC loop extruders such as Condensin, and the activity of RNA binding proteins is essential to maintain chromosome organization and stability in both interphase and mitosis. To explore the links between SMC complexes and RNA metabolism we studied the interactome of Fission yeast Condensin and found that it physically interacts with RNA binding proteins involved in RNA catabolism. Among these RBPs we found that Rrp6, a catalytic subunit of the nuclear RNA-exosome, directly binds Condensin. Controlled degradation of Rrp6 in a condensin-deficient genetic background led to loss of cell viability and to a specific missegregation of the rDNA during anaphase. Remarkably, Hi-C indicates that most of the genome remains efficiently folded in metaphase despite Rrp6 degradation, further suggesting that Rrp6 might collaborate with condensin specifically at rDNA repeats. The role played by Rrp6 and/or the RNA exosome with respect to Condensin-mediated chromosome assembly and segregation will be discussed.

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ID de Contribution: **51** Type: **Non spécifié**

A minimal in vivo model to quantify the interplay between transcription and DNA supercoiling

In bacteria, genetic context can have significant impact on gene expression but is currently not integrated in quantitative models of gene regulation despite known biophysical principles and quantitative *in vitro* measurements. Conceptually, the simplest genetic context consists of a single gene framed by two topological barriers, also known as the twin transcriptional-loop model, which illustrates how transcription both affects and is affected by DNA supercoiling. *In vivo*, DNA supercoiling is additionally modulated by topoisomerases whose modus operandi remains to be quantified.

In this talk, I will first present an experimental realisation of the twin transcriptional-loop model in *Escherichia coli*. I will then show how a first-principle biophysical model of DNA transcription accounts quantitatively for all the data and makes predictions on topoisomerase activity *in vivo*.

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ID de Contribution: **52** Type: **Non spécifié**

Coarse-grained modelling of the bacterial nucleoid

The mechanism responsible for the organization of bacterial DNA inside a structure called the nucleoid is a longstanding but still lively debated question. Most puzzling is the fact that the nucleoid occupies only a small fraction of the cell, although it is not separated from the rest of the cytoplasm by any membrane and would occupy a volume about thousand times larger outside from the cell. In this talk, I will review recent results of numerical simulations based on coarse-grained models, which elaborate on the conjecture that the formation of the nucleoid may result from a segregative phase separation mechanism driven by the demixing of the DNA coil and non-binding globular macromolecules, presumably functional ribosomes. I will also discuss the extent to which demixing collaborates (or does not collaborate) with other mechanisms that are known to play a role in the organization of the nucleoid, like supercoiling and nucleoid associated proteins.

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ID de Contribution: **53** Type: **Non spécifié**

Transcription-dependent genome folding

It is well known that the enhancer-promoter interactions are essential for gene expression, but the role of RNA Polymerase II (Pol II)-mediated activity on genome folding is remain controversial. Here by investigating Micro-C data for mESCs and Drosophila embryo, we show a significant correlation between gene compaction and Pol II occupancy inside the gene, independent of cohesin-dependent loop extrusion activity. To rationalize these observations, we develop a biophysical model for the transcription-dependent folding of the genome by coupling a mathematical description of gene transcription (binding, initiation, elongation, termination of Pol II) and a polymer model of chromosome organization integrating effective Pol II-Pol II attractive interactions. Systematic analysis of the model allows to mechanistically investigate the role of gene length, gene activity or transcriptional bursting on the spatio-temporal dynamics of gene, in agreement with experimental observations. Our work provides solid proofs that transcriptional activity shapes the 4D genome via (micro)phase separation mediated by Pol II.

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ID de Contribution: **54** Type: **Non spécifié**

Cryo-electron tomography and deep learning based image denoising reveals chromatin landscape at the nanometer resolution in situ

Folding of nucleosome chains influences DNA availability for functional interactions necessary for the regulation of transcription, DNA replication and repair. Despite the existing models based on studies *in vitro*, the nucleosome chain geometry within the crowded cell nucleus has remained elusive. Cryo-electron tomography (Cryo-ET) is the only method that provides a sufficient resolution to address this question. Our previous studies using cryo-ET of vitreous sections demonstrated the feasibility of imaging nucleosomes in Drosophila nuclei *in situ* at a level of detail that allowed us to access their conformation. In this study, combining Cryo-ET and deep learning tools, we obtained the first direct observation of the path of linker DNA in chromatin imaged directly in its functional environment *in situ*. We quantify linker length and curvature characterizing a disordered zig-zag chromatin folding motif, with a low degree of DNA bending. In addition, the quality of visualization made it possible to explore the chromatin structure directly at the level of individual nucleosome conformation without structure averaging that was used by previous cryo-ET studies. Our results on identification of nucleosome conformational transitions and non-canonical nucleosome structures *in situ*, further highlighting potential of this methodology for deciphering chromatin organization.

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ID de Contribution: **55** Type: **Non spécifié**

Chromatin state dynamics and regulation of circadian clock function.

Mammalian circadian oscillators are built on a feedback loop where the activity of the transcription factor CLOCK:BMAL1 is repressed by the PER-CRY complex. We found that Per deficient cells displayed altered nucleosome occupancy around transcription start sites (TSS) and at promoter-proximal and distal CTCF sites in the genome. This chromatin re-organization was coincident with a significant decrease in histone variant H2A.Z deposition, a process that is reversed by re-expressing mPer2. Knocking out H2A.Z altered nucleosome occupancy at clock promoters, TSS and CTCF sites and thus mimicked Per loss. H2A.Z deletion furthermore completely disrupted cellular rhythms. Our work implicates a PER-H2A.Z network hub at the core of the circadian oscillator regulating not just the establishment of the clock mechanism but also its outputs and genome organization. Thus, physio-pathological scenarios where H2A.Z is mis-expressed, or its deposition dynamics, recognition or function are mis-regulated, could potentially also be compromised for circadian function.

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ID de Contribution: **56** Type: **Non spécifié**

Kinetic modeling of nucleosome dynamics on a chromatin fibre

The eukaryotic genome is packaged inside nucleus by histone octamers that bind with DNA forming a basic repeating structural unit called nucleosomes—about 147 base-pairs (bp) of DNA is wrapped around the histone octamer. Recent evidence suggests existence of stable sub-size nucleosomes < 147 bp. This may be due to the fact that the DNA ends of the nucleosomes can unwrap and rewrap. Indeed it has been shown that transcription factors can invade/bind to unwrapped nucleosomes. Interestingly, not only nucleosome formation but also the unwrap-rewrap dynamics has been shown to depend on the underlying DNA sequence. How these sub-nucleosomes are organized in the genome and affects nucleosome occupancy and positioning is not fully understood. Here we report a preliminary theoretical study of nucleosome dynamics on some finite-size DNA. We want to know the minimal physical conditions (genome length, initial nucleosome configurations, relaxation time, and unwrapping rates) in the kinetic model such that the nucleosome occupancy per base-pair approaches to the equilibrium models.

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ID de Contribution: **57** Type: **Non spécifié**

Strategies to detect phase separation in cells

Despite the tremendous progress in studying biomolecular condensates, it remains a challenge to reliably detect and quantify condensates produced by liquid-liquid phase separation (LLPS) in living cells. In particular, liquid droplets produced by LLPS and stretches of chromosomes decorated with nuclear proteins that do not undergo LLPS can share several properties, like an appearance as 'foci'and dynamic turnover of molecules. To address this issue, we are currently developing experimental and computational strategies to distinguish both cases. On the one hand, we use half-FRAP experiments, in which half of the condensate is bleached and the intensity in both halves is followed. We find that such experiments can distinguish between a liquid droplet formed by LLPS and a scaffold that is decorated with transiently binding proteins. We show that an anti-correlation between the intensities in both halves is a robust signature of LLPS that is related to the preferential internal mixing of condensates, and we establish a quantitative relationship between this signature and the interfacial energy at the boundary of the condensate. On the other hand, we work on a computational workflow to retrieve key information about cellular condensates from published large-scale datasets, including the type and number of molecules in each condensate. We use this information to infer the biophysical nature of the condensates and to compare it to the expectations for LLPS. Both approaches are complementary to each other and will hopefully improve our understanding of biomolecular condensates in cells.

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ID de Contribution: **58** Type: **Non spécifié**

What is a supercoiling-sensitive gene? Insights from topoisomerase I inhibition in Dickeya dadantii

DNA supercoiling is an essential mechanism of bacterial chromosome compaction, whose level is mainly regulated by topoisomerase I and DNA gyrase. Inhibiting either of these enzymes with antibiotics leads to global supercoiling modifications and subsequent changes in global gene expression. In previous studies, genes responding to DNA relaxation induced by gyrase inhibition were categorised as "supercoiling-sensitive". Here, we studied the opposite variation of DNA supercoiling in the phytopathogen Dickeya dadantii using the non-marketed antibiotic seconeolitsine. We showed that the drug is active against topoisomerase I from this species, and analysed the first transcriptomic response of a Gram-negative bacterium to topoisomerase I inhibition. We find that the responding genes essentially differ from those observed after DNA relaxation, and further depend on the growth phase. We characterised these genes at the functional level, and also detected distinct patterns in terms of expression level, spatial and orientational organisation along the chromosome. Altogether, these results highlight that the supercoiling-sensitivity is a complex feature, which depends on the action of specific topoisomerases, on the physiological conditions, and on their genomic context. Based on previous in vitro expression data of several promoters, we propose a qualitative model of SC-dependent regulation that accounts for many of the contrasting transcriptomic features observed after gyrase or topoisomerase I inhibition.

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ID de Contribution: **59** Type: **Non spécifié**

In vivo chromatin signatures at nucleosome inhibitory energy barriers (NIEBs) predicted by physical model

In eukaryotes, DNA is wrapped around histones to form nucleosomes, the building blocks of chromatin. The condensation of the genome is not the only function of this organization and chromatin acts as a substrate of many processes: replication, transcription, DNA reparation. Even if most of the genome is thought to be covered by nucleosomes, physical modeling predicts some nucleosome inhibitory energy barriers (NIEBs) based on the DNA sequence. It has recently been shown that the predicted NIEBs is ubiquitous from yeast to mammals and correlate with in vivo data. Physical modeling, confirmed by in vivo results, highlights the statistical positioning of nucleosomes at the borders of those barriers. Based on MNase-sequencing and MNase-ChIP-sequencing data, we characterized the chromatin signature at NIEBs borders. We show that the model outperform initial expectation, predicting correctly nucleosomes over kilobases at the border of NIEBs. The model predicts a first nucleosome perfectly positioned follows by arrays of nucleosomes of different repeat lengths. We then highlight a very dynamic structure at NIEBs border suggesting that the energetic profile encoded in DNA sequence, and resulting from evolution, could be the base for chromatin organization and plasticity.

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ID de Contribution: **60** Type: **Non spécifié**

Uncovering dynamic genome organization by passive observation and active manipulation

The DNA in a cell's nucleus is intricately structured across 4 orders of magnitude in space. Over the past decade, our understanding of these structures has advanced tremendously, but remains constrained to static snapshots. I will discuss two recent approaches that go beyond the static picture using fluorescence microscopy in live cells. By observing the interaction of two genomic loci on the same chromosome, we were able to study the formation and dissolution of chromatin loops, which are believed to play important structural an regulatory roles. Zooming out to scales of the whole nucleus, magnetic manipulation of a single genomic locus allowed us to study the mechanical properties, specifically the force response, of chromatin in living cells. Both works add to our understanding of the dynamical behavior of chromatin, thus contributing to a full four dimensional understanding of genome organization.

References: https://www.science.org/doi/abs/10.1126/science.abn6583, https://www.biorxiv.org/content/10.1101/2021.04.20

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ID de Contribution: **61** Type: **Non spécifié**

Enhancing single molecule localization microscopy with deep learning

Single-molecule localization microscopy (SMLM) is a powerful super-resolution imaging technique that can image biological structures at near-molecular scales (resolutions down to ~20 nm or better), enabling broad applications in the life sciences. With standard reconstruction methods, SMLM typically requires ~10^4-10^5 low-resolution frames to generate a single super-resolution image, hence the temporal resolution of SMLM is very poor. To improve this, we previously developed a deep learning approach (ANNA-PALM) that reduces the number of required frames for structures such as microtubules by up to ~100-fold. However, deep learning-based approaches are susceptible to model mismatch, whereby artifacts can arise in case of inconsistencies between training and test data. An obvious approach to alleviate this issue and increase the robustness of deep learning-based methods is to increase the quantity and diversity of training data. Unfortunately, despite the widespread use of SMLM, there is comparatively little sharing of SMLM data in the community, in part for lack of dedicated tools, resulting in a dearth of training data.
To address this challenge, we developed ShareLoc (https://shareloc.xyz), an open platform designed to enable sharing, easy visualization, and reanalysis of SMLM data in accordance with FAIR principles (findable, accessible, interoperable, reusable). Thanks to the Shareloc platform, we retrained ANNA-PALM on a much larger and more diverse data set than our original model. We demonstrate empirically that data sharing through ShareLoc allows to significantly improve the quality and robustness of ANNA-PALM reconstructions when tested on images from a fifth, independent lab. More generally, we expect that ShareLoc will accelerate the development of state-of-the-art analytical techniques and the promotion of reproducible research in the field of SMLM and its numerous applications in biological research.

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Origin of DNA replication: positioning and strength, from bulk to single cell experiment

The positioning and strength of origin of DNA replication in human are still poorly defined. Origins are licensed in G1 phase and fired in S phase of the cell cycle. Experiments can independently profile mean replication timing (MRT) and replication fork directionality (RFD) genome-wide. Such profiles contain information on multiple origins'properties and on fork speed. Due to possible origin inactivation by passive replication, however, observed and intrinsic origin efficiencies can markedly differ. Thus, there is a need for methods to infer intrinsic from observed origin efficiency, which is context-dependent. Here, we show that MRT and RFD data are highly consistent with each other. Using neural networks, we infer an origin licensing landscape that, when inserted in an appropriate simulation framework, jointly predicts MRT and RFD data with unprecedented precision. We furthermore uncover an analytical formula that predicts intrinsic from observed origin efficiency combined with MRT data. While this formula has been derived using various approximation, we show that it can be exact for a specific type of firing and can be also used to study single cell experiments.

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