18th CRG SYMPOSIUM: Single cell and single molecule biology

Organized by:

CRG Systems Biology Programme

BOOK OF ABSTRACTS

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18th CRG SYMPOSIUM: Single cell and single molecule biology

PRBB Auditorium, Barcelona

14-15 November 2019

Organized by:

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CRG Systems Biology Programme

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SYMPOSIUM PROGRAMME

THURSDAY, 14 NOVEMBER

09:15-09:30 Welcome by the organizers

SESSION 1 - DEVELOPMENT, DIFFERENTIATION AND ORGANOIDS

09:30-10:00 *"Understanding the regulation of gene expression using single cell genomics"*
John Marioni

10:00-10:30 *"Regeneration in intestinal organoid development"*
Prisca Liberali

10:30-11:00 *"Single cell systems biology of adult stem cells"*
Lars Velten

11:00-11:30 Networking Café

SESSION 2 - GENE EXPRESSION AND EPIGENETICS

11:30-12:00 *"Single molecules revealing molecular mechanisms underlying microtubule cytoskeleton function"*
Thomas Surrey

12:00-12:30 *"Single-molecule epigenomics: Decoding the histone code in health and disease"*
Efrat Shema

CONTRIBUTED TALKS

12:30-12:45 *"Single-cell multi-omics CRISPR-seq will reveal the role of DNA methylation during exit from pluripotency and lineage specification"*
Tim Lohof

12:45-13:00 *"Reconstructing and visualising cell lineages"*
Irepan Salvador-Martinez

13:00-14:00 LUNCH & POSTER SESSION

SESSION 3 - TECHNOLOGY (SEQUENCING SINGLE DNA MOLECULES + SINGLE PROTEINS)

14:00-14:30 *"Single Molecule Protein Sequencing"*
Edward Marcotte

14:30-15:00 *"Single-Cell Transcriptomics: Towards a High-Quality Cell Atlas"*
Holger Heyn

15:00-15:30 POSTER SESSION CAFÉ

SESSION 4 - DIVERSITY OF LIFE

15:30-16:00 *"Single-cell regulatory genomics in the Drosophila nervous system"*

Stein Aerts

16:00-16:30 *"Single-cell analysis of early animal cell type programs"*

Arnau Sebé

16:30-17:00 *"Single cell transcriptome analysis in a proto-vertebrate and the origins of novel cell types"*

Mike S. Levine

FRIDAY, 15 NOVEMBER

SESSION 5 - CELLS AND TISSUES

10:00-10:30 *"Mapping the female reproductive tissue one cell at a time"*

Roser Vento

10:30-11:00 *"Single-cell genomic atlas of great ape cerebral organoids"*

Barbara Treutlein

11:00-11:30 NETWORKING CAFÉ

CONTRIBUTED TALKS

11:30-12:00 *"Single-molecule assays reveal the essential building blocks of a mammalian mRNA transport complex and their function"*

Sebastian Maurer

12:00-12:15 *"Single-cell genome-plus-transcriptome sequencing without upfront preamplification "*

Koen Theunis

12:15-12:30 *"Single-cell transcriptomics analysis reveals the dynamics of alternative polyadenylation during cell cycle progression"*

Mireya Plass

12:30-12:45 CLOSING REMARKS

12:45-14:00 LUNCH & POSTER SESSION

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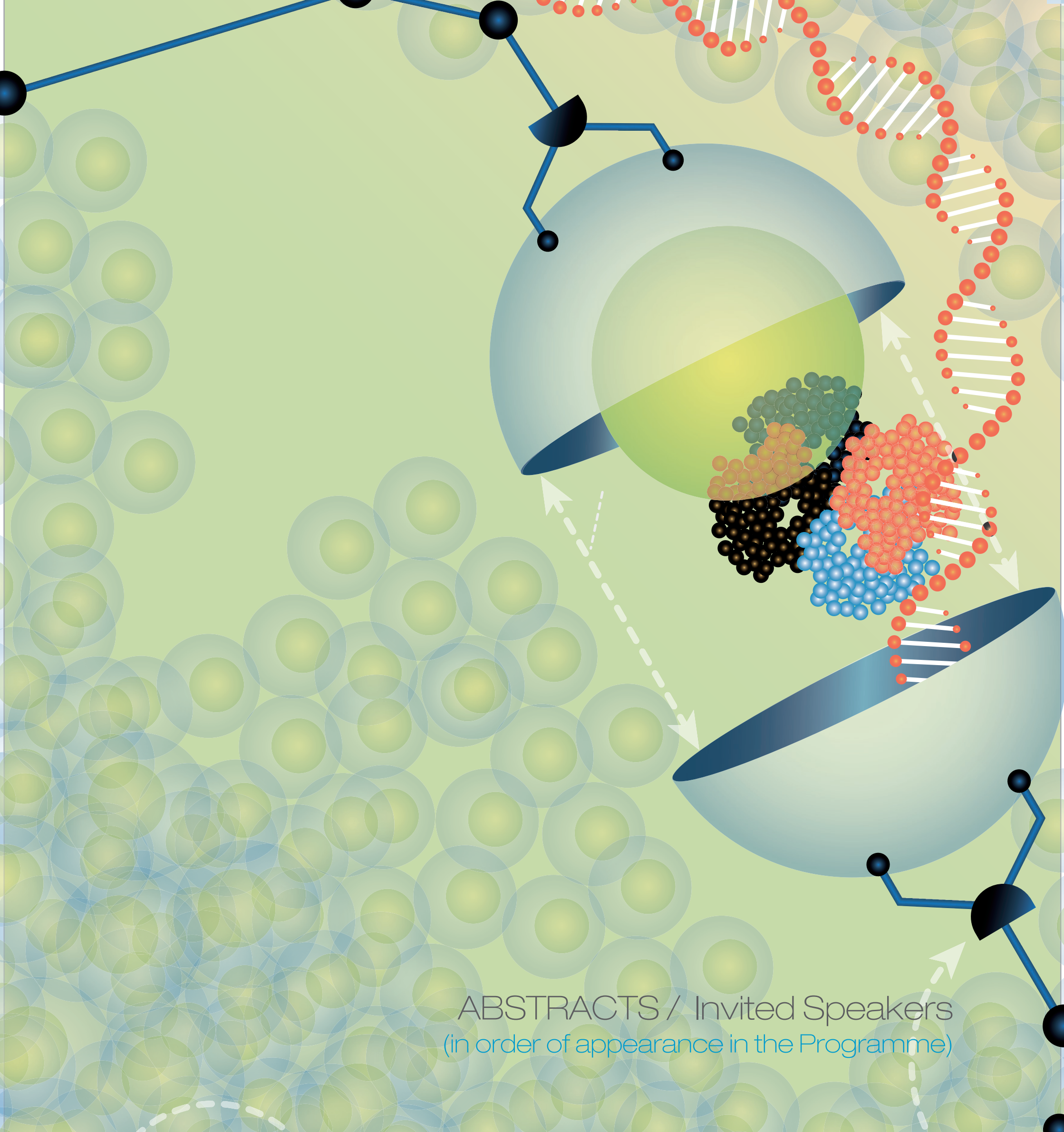

ABSTRACTS / Invited Speakers

(in order of appearance in the Programme)

*These abstracts should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with consent of the author.

Understanding the regulation of gene expression using single cell genomics

John MARIONI

European Bioinformatics Institute (EMBL-EBI) and

CRUK Cambridge Institute, Cambridge, UK

Abstract: With recent technological developments it has become possible to characterize a single cell's genome, epigenome, transcriptome and proteome. However, to take advantage of such data it is critical that appropriate computational methods are applied and developed. In this presentation, I will describe some of the computational challenges and the solutions we have developed, focusing particularly on applications in the context of cell fate decisions in early mammalian development.

Regeneration in intestinal organoid development

Prisca LIBERALI

Friedrich Miescher Institute for Biomedical Research, Basel, CH

Single-cell RNA sequencing (scRNAseq) is at the forefront of techniques to chart molecular properties of individual cells. Recent methods are scalable to thousands of cells, enabling an unbiased sampling and in-depth characterization without prior knowledge. Consequently, studies aim to produce comprehensive cellular atlases of tissues, organs and organisms.

We implemented high-throughput scRNAseq processes for different protocols (MARS-seq, Smart-seq2, Seq-well) with >50,000 single cells sequenced and analyzed to date. We are joining computational, statistical and biological knowledge in order to determine best practices in single-cell research and to relate genome activity to cellular phenotypes. A systematic comparison of different scRNAseq protocols pointed to large differences in sensitivity of molecule capture, with a high degree of accuracy across the methods. We critically enlarged the scope of such methods by establishing cryopreservation as suitable method for sample transfer and clinical archiving.

We applied single-cell transcriptome analysis for cellular phenotyping to resolved heterogeneity during developmental processes, complex tissues or tumor evolution. To address the challenges of future large scRNAseq data sets, we developed an analytical framework for the sensitive detection of population markers and differentially expressed genes, being scalable to analyze millions of single cells. Analyzing 1.3 million cells from the mouse developing forebrain, we identified rare populations of neurons, for which we determined a previously not recognized heterogeneity associated to distinct differentiation stages, spatial organization and cellular function.

Single cell systems biology of adult stem cells

Lars VELTEN

EMBL, Heidelberg, DE

Tissues are highly dynamic structures that constantly replenish themselves and autonomously organize in space. The bone marrow (BM) is a particularly fascinating tissue, since it supplies our bodies with one trillion blood cells every day, and at the same time builds and maintains our skeleton. These processes are thought to be regulated by the interaction of hematopoietic stem cells, mesenchymal stem cells, their progeny, and endothelia. Using a combination of single cell and spatial transcriptomics, we have created an atlas of cell types and their localization in BM. We clarified the differentiation trajectories of hematopoietic and mesenchymal stem cells, identified unexpected new cytokine secreting cells, and could show that their distinct spatial localization establishes niches that differentially impact stem cell activity. Importantly, we also demonstrated that the three-dimensional organization of the BM can be accurately inferred from single-cell gene expression data by exploiting information contained in the expression of cell adhesion proteins. Together, our work reveals the cellular and spatial organization of bone marrow, and offers a novel strategy to dissect the complex organization of whole organs in a systematic manner.

Single molecules revealing molecular mechanisms underlying microtubule cytoskeleton function

Thomas SURREY

Centre for Genomic Regulation, Barcelona, ES

The microtubule cytoskeleton is involved an many cellular functions. One hallmark of microtubules is their property to switch between phases of growth and shrinkage, called dynamic instability. During growth microtubules are thought to be protected by a stretch of tubulins at their very end which have GTP bound that is hydrolyzed with a delay, forming the so-called GTP cap. The size and structure of the GTP cap is not fully understood. Here we show how ensemble and single molecule imaging of end binding proteins of the EB1 family to human microtubules with tuned GTPase kinetics can provide insight into the properties of the GTP cap that ultimately determine the basic characteristics of microtubule dynamics.

Single-molecule epigenomics: Decoding the histone code in health and disease

Efrat SHEMA

Department of Biological Regulation, Weizmann Institute of Science, Israel

Different combinations of histone modifications have been proposed to signal distinct gene regulatory functions, but are poorly addressed by existing technologies. We recently published a novel high-throughput single-molecule imaging technology to decode combinatorial modifications on millions of individual nucleosomes (Shema et al., Science, 2016). We apply this technology to elucidate the epigenetic events that drive tumorigenesis in cancers carrying mutations in critical epigenetic pathways. Specifically, we study diffuse midline gliomas (DIPG), aggressive and deadly pediatric brain tumors harboring point mutations in the histone H3 Lysine 27. We combine the single-molecule proteomic platform with single-molecule DNA sequencing technology to simultaneously determine the modification states and genomic positions of individual nucleosomes. Our single-molecule epigenetic profiling technology is a transformative new tool for functional genomics with the potential to unravel the contribution of epigenetic alterations to tumorigenesis. We also harness this technology to reveal the tissue-of-origin of cell-free DNA circulating in our blood in the form of nucleosomes, and apply it to devise novel strategies for early detection of cancer and other diseases

Single Molecule Protein Sequencing

Edward MARCOTTE

1 Center for Systems and Synthetic Biology, Department of Molecular Biosciences, University of Texas at Austin, Austin, US

2 Department of Chemistry, University of Texas at Austin, Austin, US

3 Erisyon, Inc., Austin, US

The identification and quantification of proteins lags behind DNA sequencing methods in scale, sensitivity and dynamic range. Currently, mass spectrometry is the method of choice for large-scale protein identification, but it is limited in its ability to analyze low-abundance samples and map rare amino acid variants. These limitations can in principle be addressed by highly parallel single-molecule protein sequencing, a concept analogous to nucleic acid technologies that sequence millions to billions of oligonucleotides in complex mixtures in parallel. I'll describe our development of such an approach, termed fluorosequencing, for directly visualizing individual fluorescently labeled peptide or protein molecules as they are sequenced by step-wise chemical degradation. This required developing instrumentation and methods, extensive testing of fluorophores, microfluidic design, chemistry of peptide immobilization and sequential degradation, image processing algorithms for monitoring individual peptide's fluorescent intensities, and classifying and modeling the sources of errors. Using fluorosequencing, we show that sparse amino acid sequence information can be obtained for individual protein molecules for thousands to millions of molecules in parallel. Single-molecule protein sequencing combines aspects from DNA sequencing, mass spectrometry proteomics, and classic Edman sequencing, and can potentially improve the sensitivity and throughput of proteomics experiments by orders of magnitude, as well as offering digital quantification, by counting molecules.

Single-Cell Transcriptomics: Towards a High-Quality Cell Atlas

Holger HEYN
Centro Nacional de Análisis Genómico (CNAG-CRG)
Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, ES
Universitat Pompeu Fabra (UPF), Barcelona, ES

Single-cell RNA sequencing (scRNAseq) is at the forefront of techniques to chart molecular properties of individual cells. Recent methods are scalable to thousands of cells, enabling an unbiased sampling and in-depth characterization without prior knowledge. Consequently, studies aim to produce comprehensive cell atlases of healthy and diseased tissues and organs. However, there are large differences between scRNAseq techniques and it remains elusive which protocols are most adequate to draw a cell atlas. We generated benchmark datasets to systematically evaluate techniques for their power to describe cell types and states comprehensively. The methods revealed large differences in their performance, which has to be considered when defining guidelines and standards in the framework of international consortia, such as the Human Cell Atlas project. We further identified sampling biases that arise in large patient cohorts and biobank projects. We detected a systematic gene expression bias introduced during sample archiving, an important features that need to be taken into account when designing experiments for population-based association studies. Failing to select suitable samples or to correct datasets during analyses can lead to biased or false reporting.

Single-cell regulatory genomics in the Drosophila nervous system

Stein AERTS

VIB Center for Brain & Disease Research, Leuven, BE

Single-cell transcriptomics and single-cell epigenomics allow building cell atlases of any tissue and species, which can provide unprecedented insight into the dynamics of cellular state transitions during developmental or disease trajectories. I will describe recent computational methods to identify transcription factors, gene networks, and cell states from single-cell data. These methods include SCENIC for the inference of gene networks from scRNA-seq data; cisTopic for the prediction of co-regulatory enhancers from scATAC-seq data; and SCope for the visualisation of single-cell atlases. To integrate single-cell epigenome and transcriptome data we exploit cis-regulatory sequence analysis, using deep learning and large databases of transcription factor recognition motifs. In the second part of my talk I will present several data sets from the Fly Cell Atlas, with a focus on the development of the retina and the brain, where we aim to trace genomic regulatory programs of neuronal identity at single-cell resolution.

Single-cell analysis of early animal cell type programs

Arnau SEBÉ

Centre for Genomic Regulation, Barcelona, ES

A hallmark of animal multicellularity is the spatial co-existence of specialized cell types, defined by genome regulatory mechanisms that differentially interpret genetic information in every cell. Therefore, the emergence and diversification of cell types is a leading factor in animal evolution. However, so far the diversity and evolutionary dynamics of cell types has remained largely unexplored beyond selected tissues in a few species. The advent of single-cell transcriptomics and other functional genomics technologies enables the systematic dissection and comparison of whole-organism cell type programs across distant animal phyla. By applying these tools to basal metazoans (sponges, ctenophores, cnidarians), we reveal early animal cell type complexity and provide insights into the evolution of animal cell-specific genomic regulation.

Single cell transcriptome analysis in a proto-vertebrate and the origins of novel cell types

Mike S. LEVINE

Leiws-Sigler Institute for Integrative Genomics,

Princeton University, US

Quantitative live imaging methods suggest that transcriptional enhancers map far, ~200-300 nm, from their target promoters during gene activation. These and other observations are consistent with emerging evidence for "transcription condensates", higher order associations of Mediator complexes and other activators needed for gene expression. Most current analyses focus on activation, even though transcriptional repression is equally important for the patterning of the Drosophila embryo. We employed a novel assay to visualize the distribution of different sequence-specific transcriptional repressors within the nuclei of gastrulating Ciona embryos. These studies suggest that the HES repressor (segmentation and somitogenesis) is localized in droplets formed by liquid-liquid phase separation. I will discuss a simple model, whereby repression condensates sequester enhancers and their associated genes so that they are unable to engage active condensates.

Mapping the female reproductive tissue one cell at a time

Roser VENTO

Welcome Sanger Institute, UK

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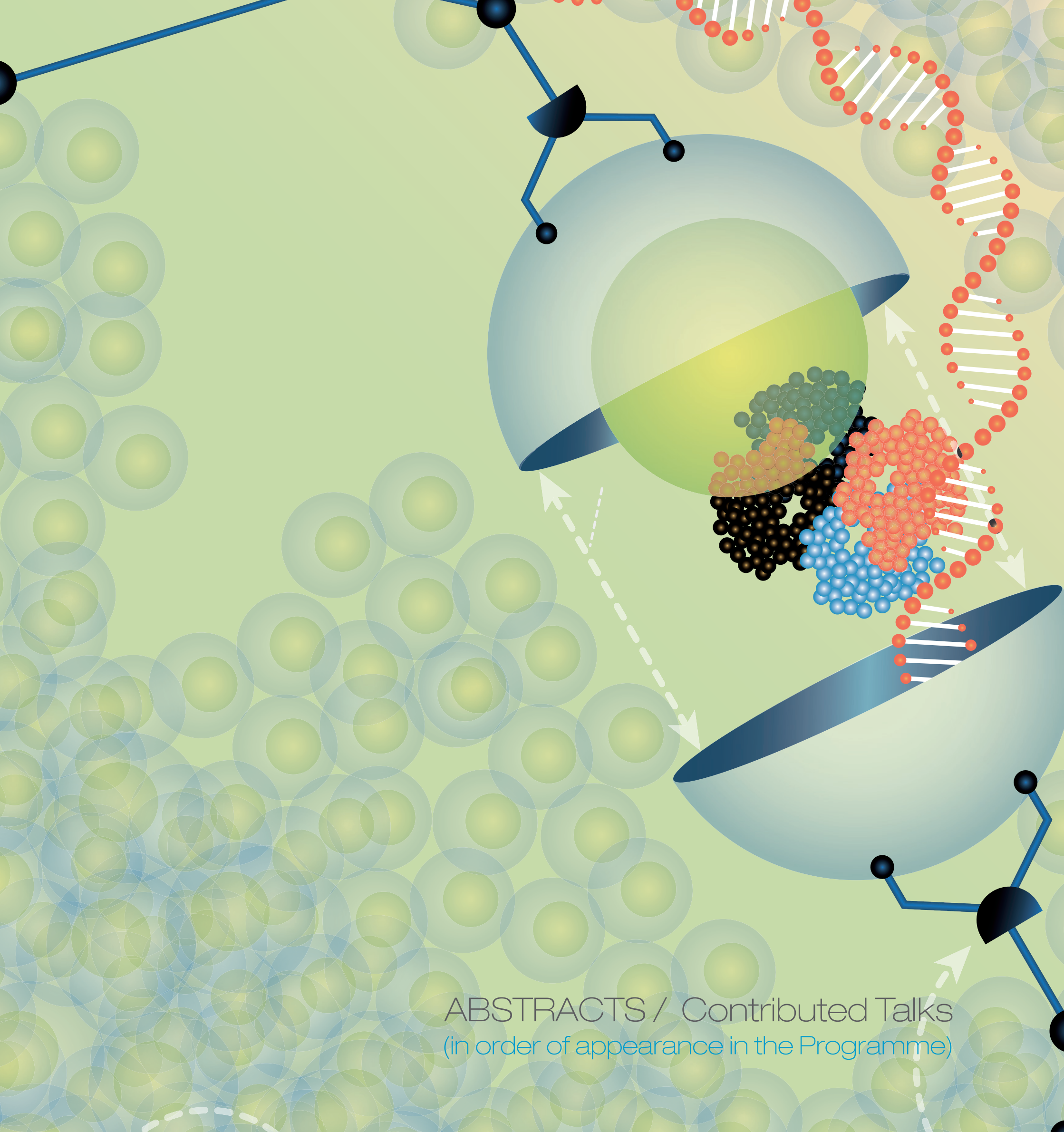
Single-cell genomic atlas of great ape cerebral organoids

Barbara TREUTLEIN

Department of Biosystems Science and Engineering, ETH Zürich, Basel, CH

The human brain has changed dramatically since humans diverged from our closest living relatives, chimpanzees and the other great apes. However, the genetic and developmental programs underlying this divergence are not fully understood. Here, we have analyzed stem cell-derived cerebral organoids using single-cell transcriptomics (scRNA-seq) and accessible chromatin profiling (scATAC-seq) to explore gene regulatory changes that are specific to humans. We first analyze cell composition and reconstruct differentiation trajectories over the entire course of human cerebral organoid development from pluripotency, through neuroectoderm and neuroepithelial stages, followed by divergence into neuronal fates within the dorsal and ventral forebrain, midbrain and hindbrain regions. We find that brain region composition varies in organoids from different iPSC lines, yet regional gene expression patterns are largely reproducible across individuals. We then analyze chimpanzee and macaque cerebral organoids and find that human neuronal development proceeds at a delayed pace relative to the other two primates. Through pseudotemporal alignment of differentiation paths, we identify human-specific gene expression resolved to distinct cell states along progenitor to neuron lineages in the cortex. We find that chromatin accessibility is dynamic during cortex development, and identify instances of accessibility divergence between human and chimpanzee that correlate with human-specific gene expression and genetic change. Finally, we map human-specific expression in adult prefrontal cortex using single-nucleus RNA-seq and find developmental differences that persist into adulthood, as well as cell state-specific changes that occur exclusively in the adult brain. Our data provide a temporal cell atlas of great ape forebrain development, and illuminate dynamic gene regulatory features that are unique to humans.



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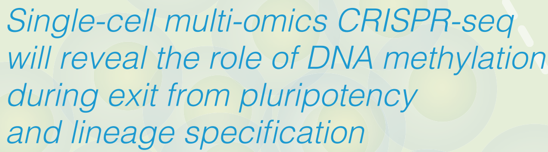


Single-cell multi-omics CRISPR-seq will reveal the role of DNA methylation during exit from pluripotency and lineage specification

Tim LOHOF

Wellcome-MRC Cambridge Stem Cell Institute,
University of Cambridge, UK

Epigenetics Programme, Babraham Institute,
Cambridge, UK

Following implantation, the mammalian embryo specifies the epiblast precursors required for the formation of the tissues of the foetus. At the exit from pluripotency, global epigenetic and transcriptional remodelling occurs. These changes are highly dynamic and known to be essential for gastrulation, the process by which all three germ layers - ectoderm, mesoderm, and endoderm - are specified. We recently used single-cell nucleosome, methylome and transcriptome sequencing (scNMT-seq) to discover epigenetic patterns associated with each germ-layer, and found a temporal asymmetry between lineages, with ectoderm methylation profiles of enhancers being established in the early epiblast, and mesoderm and endoderm profiles arising only once cells are committed to these fates. The manipulation of DNA methylation levels by knockout of methylation modifiers in vivo results in embryonic lethality shortly after gastrulation, highlighting the essential role of fine-tuned DNA methylation in mammalian development. However, the precise molecular mechanisms of how DNA methylation influences cell fate decisions are still poorly understood. In this project, we use CRISPR-Cas9 to target DNA methylation enzymes in mouse embryos and stem cell culture systems, to ask whether DNA methylation is required for particular cell fate choices. Using scNMT-seq we identify lineage-specific DNA methylation defects and by testing for correlations between molecular layers, we are able to link these methylation differences to changes in gene expression, revealing core functions of DNA methylation during cell lineage specification. In summary, this work provides a detailed study of the causal relationship between DNA methylation and cell fate in early mammalian embryogenesis.

Reconstructing and visualising cell lineages

Irepan SALVADOR-MARTINEZ
University College London, UK

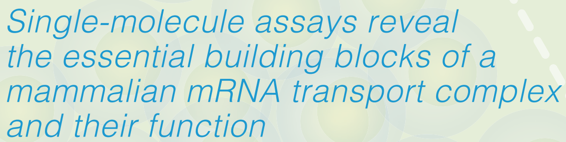
Cell lineages provide the framework for understanding how multicellular organisms are built and how cell fates are decided during development. Describing cell lineages in most organisms is challenging, given the number of cells involved; even a fruit fly larva has ~50,000 cells and a small mammal has more than 1 billion cells. Recently, the idea of using CRISPR to induce mutations during development as heritable markers for lineage reconstruction has been proposed and trialled by several groups. While an attractive idea, its practical value depends on the accuracy of the cell lineages that can be generated by this method.

First, I will show results based on computer simulations that incorporate empirical data on CRISPR-induced mutation frequencies in Drosophila. These results show significant impacts from multiple biological and technical parameters - variable cell division rates, skewed mutational outcomes, target dropouts and different mutation sequencing strategies. Our approach reveals the limitations of recently published CRISPR recorders, and indicates how future implementations can be optimised to produce accurate cell lineages.

Then I will present a web-server based software that can be used to interactively visualise cell lineages within their spatial context. This could be applicable to visualise cell lineages reconstructed by different means, from in situ sequencing of lineage recorders, to cell lineage tracking via live imaging, etc. As a proof of concept, I will present an interactive visualisation of different available data, including the cell lineage of the nematode C. elegans.

Reference:

Salvador-Martínez, I., Grillo, M., Averof, M., & Telford, M. J. (2019). Is it possible to reconstruct an accurate cell lineage using CRISPR recorders? eLife. https://doi.org/10.7554/eLife.40292



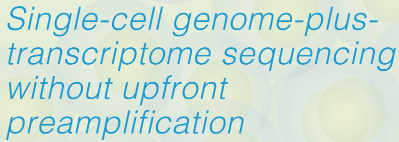
Single-molecule assays reveal the essential building blocks of a mammalian mRNA transport complex and their function

Sebastian MAURER

Group Leader, Cytoskeleton dependent RNA distribution mechanisms,

Centre for Genomic Regulation (CRG), Barcelona, ES

Through the asymmetric distribution of mRNAs cells spatially regulate gene expression to create cyto¬plasmic domains with specialized functions. In mammalian neurons, mRNA localization is required for essential processes such as cell polarization, migration and synaptic plasticity underlying long-term memory formation. The essential components driving cytoplasmic mRNA transport in neurons and mammalian cells are not known. Here we us a combination of in vitro reconstitutions and Total-Internal-Reflection-Microscopy assays with single-molecule sensitivity to directly visualise and dissect the machinery and regulatory mechanisms driving mammalian mRNA transport. Our reconstitution shows that the tumour suppressor adenomatous polyposis coli (APC) forms stable complexes with the axonally localised beta-actin and beta2B-tubulin mRNAs which are linked to a heterotrimeric kinesin-2 via the cargo adaptor KAP3. APC activates kinesin-2 and both proteins are sufficient to drive specific transport of defined mRNA packages. Guanine-rich sequences located in 3'UTRs of axonal mRNAs increase transport efficiency and balance the access of different mRNAs to the transport system. Our findings hence reveal for the first time a minimal set of proteins capable of driving kinesin-based, mammalian mRNA transport.



Single-cell genome-plus-transcriptome sequencing without upfront preamplification

Koen THEUNIS

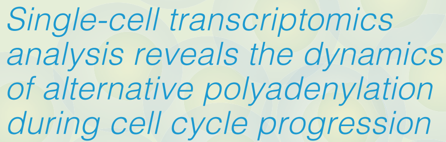
Laboratory of Reproductive Genomics, Department of Human Genetics, KU Leuven, Leuven, BE

Single-cell sequencing is a powerful tool to investigate the biology of cellular heterogeneity and is revolutionizing different fields of life sciences. Recently, single-cell multi-omics technologies have emerged, which enable profiling more than one omics layer of the same cell and thus the interplay between the layers. Genome-plus-transcriptome sequencing (G&T-seq), for instance, enables reconstructing DNA-based cell lineage trees that can be annotated with RNA-based cell type and state information, and is able to reveal the functional consequence of acquired DNA alterations in a single-cell. To sequence a single-cell, whole-genome amplification (WGA) or whole-transcriptome amplification procedures and subsequent library preparation are currently still the standard. Methods relying on single-cell preamplification and library preparation are typically time consuming and expensive.

Therefore, we developed Gtag&T-seq, a novel G&T-seq protocol, that prepares preamplification-free genome and transcriptome sequencing libraries from the same cell. Following separation of the DNA and RNA, the DNA is prepared for sequencing using direct tagmentation (Gtag), hereby avoiding upfront WGA. We evaluated the performance of DNA-copy number analysis after Gtag to conventional picoPlex after G&T separation. We applied Gtag&T-seq to 126 single-cells from a cancer cell line and a normal cell line, and to 176 single-cells from a human melanoma PDX model. The number of cells processed by conventional G&T was respectively 86 and 176 single cells.

Estimation of DNA copy-numbers in the diploid cell line was as accurate for both methods (Gtag versus picoPlex). However, Gtag was more accurate in estimating DNA copy-number states in the polyploid cancer cell line and cells from the primary tissue. Additionally, the cost of Gtag is over an order of magnitude lower than picoPlex.

In conclusion, Gtag&T-seq is a novel, low-cost, and accurate single-cell multi-omics method that enables the exploration of somatic genetic alterations and their functional consequences in single cells at scale.



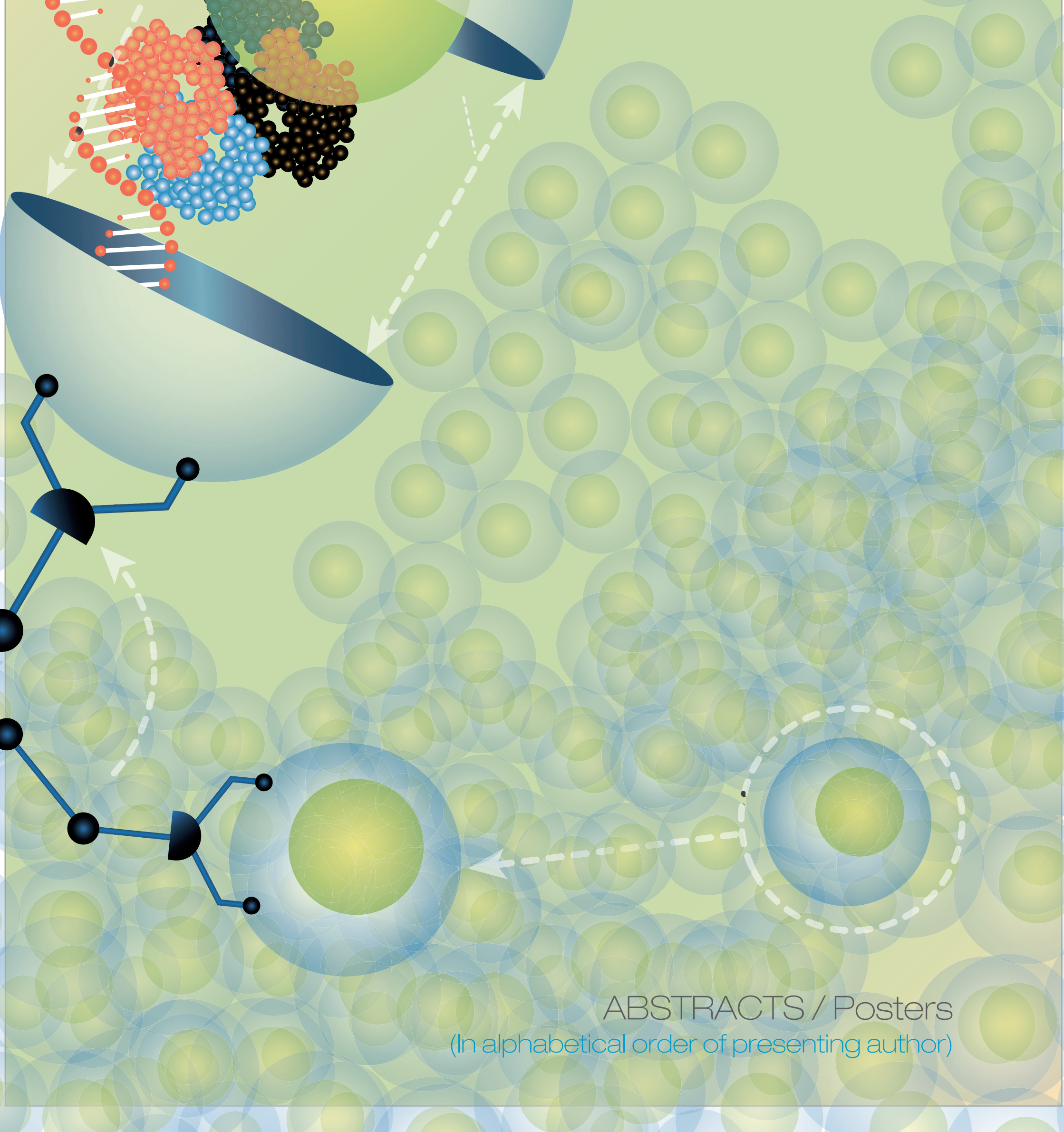
Single-cell transcriptomics analysis reveals the dynamics of alternative polyadenylation during cell cycle progression

Mireya PLASS

Systems Biology of Gene Regulatory Elements,
Berlin Institute for Medical Systems Biology, Max
Delbrück Center for Molecular Medicine, DE

Single-cell transcriptomics has revolutionized the way we can study the dynamics of gene regulation and its impact in cell proliferation and differentiation. However, specific methods to quantify transcript isoforms at the single-gene level are lacking. This prevents the exploration of dependencies that may exist across gene isoforms and a deeper understanding of how different post-transcriptional regulatory processes such as splicing and polyadenylation are coregulated.

We have developed a new method to quantify the expression levels of gene isoforms produced using insert size variation Drop-seq (isv-Drop-seq). Using this method, we have been able to quantify the thousands of individual isoforms generated by alternative polyadenylation and study their variability across cells. Additionally, we have developed another method to computationally sort cells along the cell cycle and study the dynamics of these isoforms. Our preliminary results show that many oscillating genes use specific 3' isoforms in different cell cycle phases. Together, these results suggest that the choice of 3'UTR could be related to the observed changes in expression levels of oscillating genes.



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Deoxyribozyme-based gene therapy agents against Coxsackievirus

K.P. CHALENKO, M.A. Misyurina, A.S. Volobueva, V.V. Zarubaev, E. I. Koshel, D.M. Kolpashchikov

[1] Metabolon Inc., Durham, North Carolina, USA

RNA-cleaving Deoxyribozymes (Dz) are DNA strands with RNA-cleaving catalytic activity1. In this study, we develop a Dz gene therapy agent for inactivation Coxsackievirus's RNA. IRES mRNA was chosen as a target molecule2. A universal cleavage site was found based on Coxsackie virus genome analysis of a large number of serotypes. Two synthetics RNA1 and RNA 2 (Figure 1) were synthesized and tested for cleavage by Dz1 and Dz2, respectively.

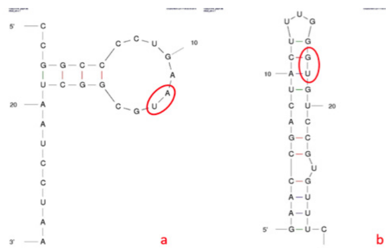

Figure 1 – The 2 secondary structures of RNA1(a) and RNA2(b).

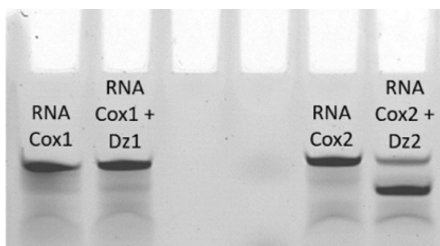

Figure 2 - Results of cleaving RNAs(1µmol) by Dz1(100nmol) and Dz2(100nmol) after 15% PAGE.

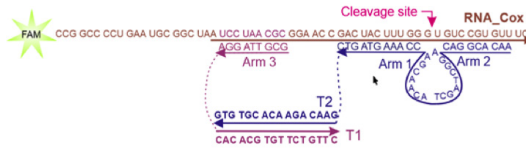

Figure 3 – Complex of RNA with Dz-based construction.

Dz2 cleaved 61,6% of RNA2, which was 7 times greater than 8,7% of RNA1 cleavage by Dz1 after 24 hrs of incubation (Figure 2). Dz2 therefore was chosen for ex vivo experiments. Additional arm(3) was added to improve unwinding RNA (Figure 3). Experiments was conducted with eukaryotic cells infected by Coxsackievirus B3, serotype Nancy. Constuctions was delivered using transfection by lipofectamine 2000. Results Dz2 treatment demonstrate 10 times reduced viruses load. Moreover, gene therapy agent with 2 additional arms was created based on Dz2, and expected to be more efficient than Dz2 with one arm. Our results indicate that Dz-based gene therapy agents have the potential to create a versatile remedy for the most Coxsackie serotypes.

References:

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Cell type comparison in larvae and medusae of the hydrozoan Clytia hemisphaerica

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This project is part of the framework of the Marie Sklodowska-Curie Innovative Training Network 'EvoCELL'. The aim of the network is to generate and compare single cell transcriptomes across animal phyla to address animal cell type evolution, as well as particular biological questions.

Using the laboratory model hydrozoan species Clytia hemisphaerica, the main purpose of this study is to define transcriptional profiles of individual cell types in both larval and adult stages exploiting single-cell RNA-sequencing technology.

These data will be the starting point for building cell types atlases, in order to investigate commonalities between the two stages and to shed light on the understanding of the complex hydrozoan life cycle, characterized by the alternation of a polyp phase and a medusa form. Furthermore, Clytia is particularly advantageous to address questions about the origin and evolution of the neurosensory system and muscles, characteristics of the medusa form, which are not present in other cnidarian species.

During the first year of my PhD I have established cell dissociation protocols for Clytia larvae and medusae and I collected single-cell transcriptome dataset of live dissociated cells for both stages using 10X Genomics platform. The data obtained have been processed using best practice bioinformatics pipelines for scRNAseq allowing identification of cell clusters and cluster-specific marker genes. I am currently validating these data via hybridization techniques in order to produce cell types atlases for both life stages.

scRNA-seq data interpretation - Computational Biology for Drugs Discovery

KOTELNIKOVA E, Ishkin A., Ivliev A. Clarivate Analytics

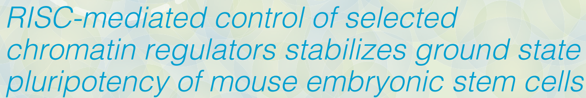
Recent technological advances allow obtaining transcriptome-wide gene expression profiles from individual cells promoting progress in biomedicine and drug discovery. To biologically interpret scRNA-seq data, various algorithms should be integrated into the workflow that brings together OMICs data and structured “knowledge” databases of networks and pathways. Development of such a workflow from scratch requires significant time and efforts.

To address this issue, Clarivate Analytics, provider of systems biology tools and databases (MetaCore, MetaBase), launched “Computational Biology Methods for Drug Discovery” (CBDD) program focused on implementation of advanced state of the art approaches for network analysis of OMICs data. CBDD is implemented in a form of modular R package that can be easily used to speed up the development of data interpretation workflows. These modules are extensively tested, described and optimized to keep the runtime low.

In CBDD there are 6 algorithms specifically designed for single-cell data:

- netSmooth – network-based algorithm for single cell dropout imputation [PMID: 29511531]
- PAGODA - analysis of ‘overdispersed’ pathways differentially active between cell subtypes [PMID: 26780092]
- SCENIC - analysis of transcription regulators activity across cell subtypes [PMID: 28991892]
- ACTION - analysis of subnetworks preferentially activated in cell subtypes [PMID: 29666373]
- SCODE - ODE-based regulatory network model reconstruction [PMID: 28379368]
- Ocone - ODE-based network model reconstruction workflow that allow more complex dynamics. [PMID: 26072513]

Most of other CBDD modules aiming on node, subnetwork or pathways interpretation, can also be applied to the gene lists derived from any single-cell analysis. Even though CBDD is not freely available, service based CBDD workflow development might be a fast and cost-efficient solution for the research labs and companies that do not need regular access to Clarivate content but would profit from it for the specific project.



RISC-mediated control of selected chromatin regulators stabilizes ground state pluripotency of mouse embryonic stem cells

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Background: Embryonic stem cells are intrinsically unstable and differentiate spontaneously if they are not shielded from external stimuli. Although the nature of such instability is still controversial, growing evidence suggests that protein translation control may play a crucial role.

Results: We performed an integrated analysis of RNA and proteins at the transition between naïve embryonic stem cells and cells primed to differentiate. During this transition, mRNAs coding for chromatin regulators are specifically released from translational inhibition mediated by RNA-induced silencing complex (RISC). This suggests that, prior to differentiation, the propensity of embryonic stem cells to change their epigenetic status is hampered by RNA interference. The expression of these chromatin regulators is reinstated following acute inactivation of RISC and it correlates with loss of stemness markers and activation of early cell differentiation markers in treated embryonic stem cells.

Conclusions: If this suggests a primary mechanism of miRNAs in preserving ES cell pluripotency and inhibiting the onset of embryonic differentiation programs, on the other hand miRNA- mediated control of chromatin regulators might maintain cells in a metastable state, which could rapidly be converted into priming to cell differentiation upon the removal of stemness-sustaining factors. Accordingly, priming could be seen as a process in which two epigenetic mechanisms are layered one on the other :a first layer, which is microRNA-mediated, on which lies a second layer consisting of chromatin-based modulation of transcription.
Single-cell transcriptomics can help to study the transition states and cell fate decision in these “Waddington epigenetic landscapes”.

Molecular profiling of ETV6-RUNX1-positive pediatric leukemia using scRNA-seq

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Purpose: Despite the relatively good prognosis of many childhood leukemia subgroups, some patients still relapse owing to drug resistance of single cells and clones. Understanding of the molecular features of leukemic blasts would aid in development of more effective targeted treatments. ETV6-RUNX1 is the most common recurrent translocation in pediatric acute lymphoblastic leukemia and results in deregulated transcription of target genomic regions by the produced abnormal transcription factor fusion.

Methods: Single cells from diagnostic or residual disease precursor-B acute lymphoblastic leukemia bone marrow samples were isolated and their transcriptional landscape inspected using the 10X Chromium single-cell RNA sequencing system. The study included six diagnostic ETV6-RUNX1-positive pre-B-ALL cases, two cases 15 days after treatment, and two normal bone marrow samples. Whole-genome sequencing was acquired from the same patients to elucidate additional genetic changes. Direct targets and markers were explored using a cell line model. The analysis was accompanied with global run-on sequencing data (active gene and enhancer regions) from the same patients.

Summary: Preliminary analysis revealed marker genes for improved segregation between HSC, pro- and pre-B-cells, which enabled comparison of pre-B-leukemic blasts with relevant reference populations. Distinct cellular phenotypes could be identified from leukemic cells carrying the same initial lesion. To our surprise, we did not detect stemness markers but rather a more differentiated phenotype in the surviving minimal residual cells after 15 days of treatment. Our analysis revealed

altered levels of B-cell differentiation pathway markers relative to diagnostic clones. Especially, CD20 (MS4A1) expression was enriched or acquired during treatment which was confirmed by anti-CD20 flow cytometry.

Conclusion: Our results indicate that ETV6-RUNX1 leukemias display heterogeneity in their molecular and cellular phenotypes that may influence the treatment response.

Topographic, single-cell gene expression profiling of Periaqueductal Gray neurons

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The midbrain periaqueductal gray (PAG) is a longitudinal columnar structure where instinctive behaviours as diverse as escaping from predators, vocalising, freezing, and pup grooming segregate onto distinct anatomical subdivisions. This relationship between behaviour and circuit anatomy provides a unique opportunity for determining how neural mechanisms support the computation of adaptive actions. Our goal is to determine how behaviourally relevant biophysical and computational properties of single PAG neurons are constrained by their gene expression profile. To achieve this, and to link the expression of ion channels, receptors, and molecular effectors to the role that PAG neurons play in the implementation of behaviours controlled by specific circuit subdivisions, we have established a pipeline to carry out single-cell RNA-sequencing while preserving the anatomical origin of each neuron. We have used visually guided aspiration via patch pipettes to isolate fluorescently labelled neurons from acute midbrain slices of transgenic mice, together with Smart-seq2 and deep sequencing to obtain detailed gene expression profiles from inhibitory and excitatory neurons of the different PAG subdivisions. Our data identify molecular signatures of specific biophysical properties, reveal the expression pattern of receptors for neuropeptides and neuromodulators that regulate neural computations, and provide testable hypotheses about molecularly defined circuit motifs that might underpin behavioural control by the PAG.

Insights into haematopoietic stem cell ageing and disease from single cell studies

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Ageing of the haematopoietic system is directly correlated with a gradual decrease in stem cell function and increase in DNA damage. In young individuals there is a tight balance between production of myeloid and lymphoid lineage cells. However, with age, there is a bias towards the myeloid lineage, leading to reduced immune response. At the same time, there is an increase in haematopoietic stem cells (HSCs) that harbour mutations in leukaemia-related genes, displaying a survival advantage. This Age-Related Clonal Haematopoiesis (ARCH)1 has been observed both in healthy and diseased aged individuals.

The same factors affect age-related disorders such as Myeloproliferative Neoplasms (MPNs), a highly heterogeneous group of pre-neoplastic diseases characterised by an abnormal increase in mature myeloid cells. The most prevalent mutation is JAK2V617F, present in 70% of MPN patients and one of the most prevalent ARCH mutations2. However, there is a high degree of phenotypic heterogeneity among patients, suggesting that the sole presence of this mutation is not enough to trigger disease initiation and progression.

To unravel other cell autonomous mechanisms leading to or preventing disease, we have designed a single cell study to identify and bharacterize old HSCs from MPN patients and healthy aged donors with high and low burden mutational profile. We are index sorting HSCs into 96-well plates to perform:

1. Single-cell culture and JAK2V617F sequencing of single-HSC clones to correlate mutational profile with growth kinetics analysis.

2. Single-cell RNA sequencing using TARGET-seq method3 to precisely identify JAK2V617F mutated cells and link mutational to transcriptional profile at the single cell level.

Gene network reconstruction using single cell transcriptomic data reveals key factors for autophagic process

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We present a novel algorithm called TENET to infer gene regulatory networks (GRNs) from single cell RNAseq (scRNAseq) data. Despite various efforts to infer GRNs, systemic attempts so far suffer from critical limitation of being applicable for the networks of handful of genes. The inferred networks have been even limited in predicting limited number of target genes.

TENET quantifies causal relationships between genes in a genome scale by applying transfer entropy (TE) to scRNAseq data. TE measures the amount of directed information transfer between two variables while considering the past events. We assessed TENET using various ways including ChIP-seq and knock-down experiments. TENET showed superior performance compared with other competitors.

We further applied TENET in dissecting the autophagic process induced by glucose starvation (GS) and amino acid starvation (AAS) in mouse embryonic fibroblasts. Strikingly, TENET successfully identified the key GS as well as AAS specific regulators. Knockdown of key factors dramatically dampened the autophagic process in a condition specific way, confirming that TENET can identify key regulators as well as their target genes. Our results provide the first evidence of distinct GRNs between AAS and GS induced autophagy. Compared with other systemic approaches to infer GRNs, TENET can be scaled enough to capture key regulators using scRNAseq. Applying TENET to other scRNAseq data, we successfully identified key regulators during developmental processes. TENET is publicly available at https://github.com/neocaleb/TENET.

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