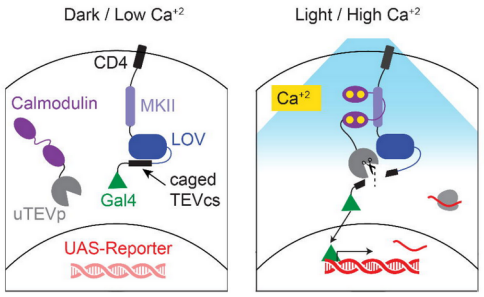
Single Cell Transcriptomics

Newsletter November 2020

Paper 1.

A molecular calcium integrator reveals a striatal cell-type driving aversion

Kim et al., *bioRxiv*, 2020.



Calcium is one of the major secondary messengers in the cells and is involved in processes such as cardiomyocite contraction, immune cell activation or release of synaptic vesicles in neurons. However, tools to measure calcium activity are mostly limited to expensive imaging set-ups with possibility to analyze only small number of cells simultaneously.

To overcome these downsides high-throughput genetic tools were developed where calcium activity is measured indirectly by the transcriptional activation of immediate early genes. Temporal control of measurement is achieved by coinciding calcium activity with the presence of artificial compounds in the cell. However, due to long diffusion/metabolization times of such compounds measurement of calcium activity suffers from low temporal resolution of ~6h.

Thus, a new genetic tool called FLiCRE (Fast Light and Calcium-Regulated Expression) was developed. Temporal control of measurement is achieved by the exposure of cells to the light. Light sensitive domains in engineered proteins change their conformation allowing binding of calcium-calmodulin complexes formed during cellular activation. Calmodulin is also fused to the protease that cleaves transcription factor that further drives transgene expression used for the readout of calcium activity. Temporal control is achieved using light stimulation, thus resolution of calcium activity measurement is within minutes. While, transgene expression as a readout allows integration of this tool with single cells sequencing.

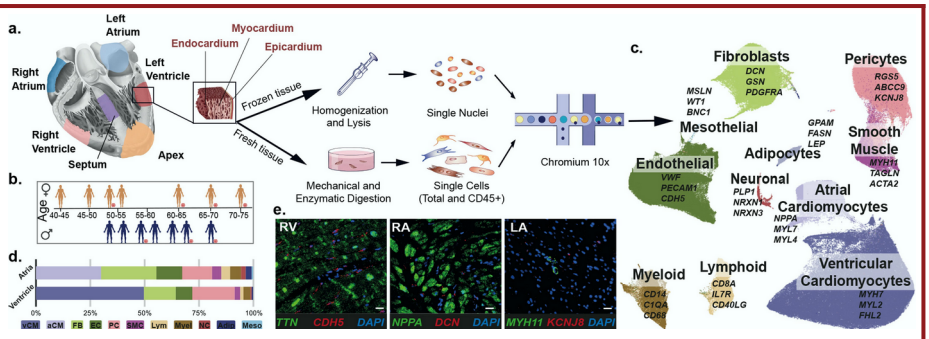
Indeed, investigators have shown they are able to identify novel functional types of neurons in nucleus accumbens in mouse brain involved in animal behavior by combining FliCRE with single cell sequencing. Moreover, light or chemically sensitive receptors/ion channels can be expressed in the identified cells allowing later direct and precise manipulation of cells involved in physiological processes or animal behavior. This is powerful technique bridging the gap between molecular and system/physiology levels and brings closer the ultimate multiomics approach we all look forward to seeing in future.

Not without downsides, this tool still suffers from the low temporal resolution of minutes while calcium activity happens on a scale of milliseconds. Moreover, such a measurement represents only a single snapshot of calcium activity and suffers from high background signal in the highly active cells. So it is still early to throw away calcium imaging gears.

Paper 2.

Cells of the adult human heart

Litviňuková et al., *Nature*, 2020.

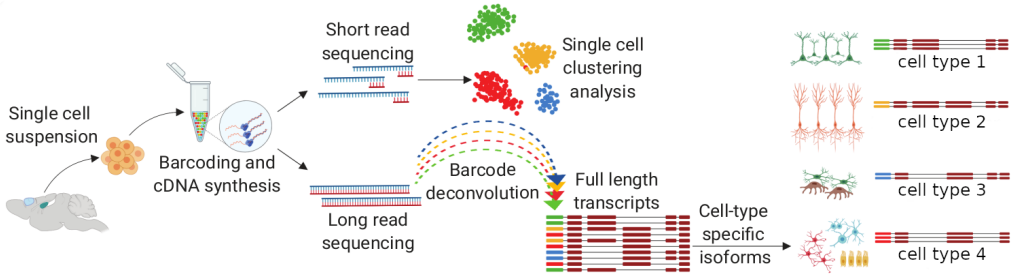


A new study focused on single cell sequencing of human heart was recently published. Larger cohort of fourteen live and healthy human hearts was obtained from UK and US donors. Availability of live heart tissue allowed investigators to perform sequencing of ~0.5 million cells (enriching endothelial and immune cells) and nuclei (to capture large and extended cardiomyocytes) using Chromium v2 and v3 chemistries. Investigators characterized six anatomical adult heart regions highlighting the cellular heterogeneity of cardiomyocytes, pericytes, and fibroblasts, and revealed distinct atrial and ventricular subsets with diverse developmental origins and specialized properties. Authors characterized the complexity of the cardiac vasculature and its changes along the arterio-venous axis, and also identified cardiac resident macrophages with inflammatory and protective transcriptional signatures. Further, inference of cell-cell interactions highlighted different macrophage-fibroblast-cardiomyocyte networks between atria and ventricles. This work provides healthy human cardiac cell atlas and improves understanding of the human heart cellular and molecular composition.

Papers 3

Cell-type, single-cell, and spatial signatures of brain-region specific splicing in postnatal development

Joglekar et al., *BioRxiv*, 2020.



The most widely-used scRNA-seq tools are often based on sequencing of one of the ends of mRNA molecule leading to the inability to assess splicing isoforms of mRNAs. However, RNA splicing is known to play important roles in cellular identity, tissue development and pathology, thus being a blind spot in the major single cell approaches. mRNA splicing investigation is further aggravated by the inability of dominant next generation sequencing technology (Illumina) to sequence long RNA reads. Thus, splicing research is often based on the reassembly of full-length mRNA isoforms (~2kb) from short (usually 35-300 bp) reads, the process often hampered by errors and low mRNA coverage in single cell data.

To overcome these limitations, authors previously developed ScISOr-Seq. First stages of the technique are based on the regular 10x 3' RNA-seq protocol. After cDNA synthesis fraction of it is used in library preparation for short-read Illumina sequencing needed for the identification of transcriptome profiles of cell subtypes. While another fraction of mRNA is used for PacBio sequencing. Although, PacBio technology has significantly lower throughput with higher costs per read comparing to Illumina sequencing, it allows sequencing of the full-length mRNA molecules crucial for splicing analysis. Thus, current protocol combines strong sides of both Illumina and PacBio sequencing, allows single cell transcriptome and spice isoform assessment, and in principle can be applied to any type of cells.

By applying ScISOr-Seq on developing mouse brain authors produced first single cell differential isoform expression (DIE) assessment of developing mouse cortex and hippocampus. They detected hundreds of brain-region specific DIE events traceable to specific cell-types. Many DIE events corresponded to functionally distinct protein isoforms. In most instances, one cell type was responsible for brain-region specific DIE. Cell types indigenous to only one anatomic structure displayed distinctive DIE. However, for some genes, multiple cell-types are responsible for DIE in bulk data, indicating that regional identity can, although less frequently, override cell-type specificity. Authors also validated their findings using modified 10x Visium protocol to perform long read sequencing for splice isoform quantification. In conclusion, this paper provides a guideline for quantifying splice isoform expression with cell-type and spatial resolution, and can be applied to any other tissue.

Next Single Cell Seminar

27th November 2020, 09:00-10:00, zoom

Holger Heyn, CRG Barcelona, Spain

Towards a high-quality human cell atlas: Applications and Guidelines

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