Chicken Cell Atlas: Science and DeSci

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Abstract
The chicken cell atlas project aims to chart the types and properties of all chicken cells across all organs and tissues, build a reference map of the mature and developing chicken bodies, and provide the resources for studying the biology of this species. It will potentially become an international collaboration among many researchers. This project will be useful for understanding the basic biology of chicken and other birds. The atlas will include spatial and temporal single-cell data on multiple modalities such as transcriptome, proteome, epigenome, glycome, metabolome, electrophysiology, morphology, and connectome. The possibility of establishing a decentralized organization based on cutting-edge crypto-technologies is discussed to create an armamentarium and organize research resources and incentives.

Chickens are not only widely consumed for their eggs and meat but are also used as a model species for biological and medical research [Stern, 2005; Burt, 2007; Haniffa et al., 2021]. To advance our understanding of this species, we need to create a cell atlas of everything representing chicks and chick embryos [Yamagata, 2022]. Similar to the mouse and human cell atlas enterprises in progress (see below), this project will generate single-cell transcriptome data for chickens, characterize each cell type, and provide foundational information integrating molecular, spatial, and temporal modalities. It will facilitate fundamental studies of chickens and other birds, including cell biology, molecular biology, developmental biology, neuroscience, physiology, oncology, virology, behavior, ecology, evolution, and animal husbandry.

The current state of the chicken cell atlas

Recent advances in single-cell RNA sequencing (scRNA-seq) have had significant effects on the study of complex tissues, leading to the discovery of novel cell types, cell states, and biomarkers [Stuart and Satija, 2019; Luecken and Theis, 2019; Alfieri et al., 2022; Zeng, 2022]. The scRNA-seq technology has opened up a plethora of opportunities to perform novel studies using new and classic model animals, including chickens (*Gallus gallus*) [Yamagata, 2022] and other birds [Colquitt et al., 2021]. A chicken cell atlas project (aka, Tabula Gallus) has been proposed to create a cell atlas of all tissues in the mature and developing chicken [Yamagata, 2022]. Like several ongoing cell atlas projects (see below), the chicken project will collect scRNA-seq data for chickens, characterize each cell type, and eventually make available information that integrates diverse modalities.

The chicken cell atlas is still in its infancy (Table 1). Nonetheless, a couple of pioneering studies have realized this endeavor, revealing various cell types and their states in the chick limb buds [Feregrino et al., 2019; De Lima et al., 2021], the early primitive streak stage [Vermillion et al., 2018; Guillot et al., 2021], the neural crests (Morrison et al., 2017; Gandhi et al., 2020; Williams et al., 2022), and the neural retina [Hoang et al., 2020; Tegla et al., 2020; Yamagata et al., 2021]. Most studies have used embryonic or juvenile tissues, likely due to accessibility. It is practicable because hatched chicks are highly active and generally considered mature. It takes 21 days, on average, for an egg to hatch once incubation begins. However, embryos are often not consistently developed, thus staged according to the Hamburger and Hamilton series [Hamburger and Hamilton, 1951]. Interestingly, in single-cell analysis, one embryo consists of a series of cell types and their states covering different developmental stages. Therefore, making multiple developmental atlases at close time points is not essential. In contrast, auxiliary atlases must be generated reflecting different factors such as sex [Clinton et al., 2001] and variable genetic background [Núñez-León et al., 2019].

The raw data from and references to scRNA-seq studies are searchable at NCBI's GEO database (https://www.ncbi.nlm.nih.gov/geo/) and several single-cell reference sites (e.g., https://panglaodb.se/papers.html, https://www.nxn.se/single-cell-studies/). To explore the original datasets, some interactive viewers for single-cell data are available (Single Cell Portal, https://singlecell.broadinstitute.org; UCSC Cell Browser, https://cells.ucsc.edu; EMBL-EBI, https://www.ebi.ac.uk/gxa/sc/home). Thus, the first endeavor toward the chick cell atlas project will be to create an armamentarium, incorporate multimodal data, display those datasets and assist users. Tabula Muris, Tabula Sapiens/Human Cell Atlas (HCA) /HuBMAP, Tabula *Drosophilae,* and C elegans atlas are examples of other species [Tabula Muris et al., 2018; HuBMAP Consortium, 2019; Haniffa et al., 2021; Taylor et al., 2021; Lindeboom et al., 2021; Eraslan et al., 2022; Li et al., 2022; Tabula Sapiens et al., 2022]. Species-specific community websites like GEISHA [http://geisha.arizona.edu] and Chickspress [https://geneatlas.arl.arizona.edu/] may provide vital starting points as a resource for chicken as in the cases for other model species. However, in the coming years, emerging crypto-technologies

such as peer-to-peer data storage and smart contract protocols could transform data sharing methods (see below).

Next steps: multimodal, spatial, and temporal atlases

In addition to scRNA-seq and relevant single-nucleus RNA sequencing, other multimodal single-cell technologies, which simultaneously profile multiple data types in the same cell, represent a frontier for discovering new cell types and characterizing cell states [Stuart and Satija, 2019]. The additional modalities include epigenome, proteome, glycome, metabolome, electrophysiology, morphology, and connectome [Guo et al., 2021, Lee et al., 2021; Saunders et al., 2021; Sun et al., 2021; Mund et al., 2022].

Among those modalities, a series of single-cell sequencing methods for detecting heritable DNA methylation and altered chromatin configurations allow the description of epigenetic changes on a genome-wide scale. In particular, a single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) is the most commonly used method for studying epigenetic landscapes in single cells [Stuart and Satija, 2019; Armand et al., 2021]. Although scRNA-seq and scATAC-seq are different, both represent the activity of genes. Thus, it is also vital to analyze proteomics and metabolomics to understand actual cell function.

The data gained from scRNA-seq and other dissociated protocols lead to the loss of spatial information. By contrast, spatial biology and spatial transcriptomics include histological, cellular, and subcellular information of transcripts in spatial context and coordinates [Rao et al., 2021; Zhuang, 2021; Palla et al., 2022; Moffitt et al., 2022; Chen et al., 2022; Moses and Pachter, 2022]. The type and function of cells are further designated by cell morphology and cell interactions, including neuronal connectivity. eCHIKIN (electroporation- and CRISPR-mediated Homology-Instructed Knock-IN) is a technique for CRISPR-mediated genome editing in somatic cells to insert GFP or Cre cDNA into genes identified as cell-type specific in scRNA-seq data [Yamagata and Sanes, 2021]. This technique will reveal cell morphology and connectivity and potentially describe the molecular and spatial networks that organize the proteome [Cho et al., 2022]. These post-transcriptional modalities, together with spatial and temporal information, facilitate the resolution of cell types and states and provide more critical information on cell function.

Open science and DeSci

In order to promote any scientific research, it is a prerequisite to have a supportive community, raise funds, and build facilities. Furthermore, all scientific data should be made openly accessible and maintained permanently. Nonetheless, most of the current "big science" projects have suffered from several drawbacks. For example, not all contributions and data submitted by individuals or institutions are satisfactorily credited. Instead, the management is often exceedingly centralized: only a handful of influential scientists are highly recognized as leaders of successful projects.

Decentralized science (DeSci) is an emerging movement that proposes to build a shared infrastructure for disseminating, assessing, funding, crediting, and storing data and knowledge using blockchain technologies [Hamburg, 2021; https://ethereum.org/en/desci/]. It aims to create an ecosystem where all the researchers are motivated to share their data and receive credit for their effort while allowing everyone open access to research materials using crypto- technologies such as non-fungible tokens (NFTs). More important, scientific organizations can be governed using tokenized incentive structures without powerful leaders by establishing decentralized autonomous organizations (DAOs). DAOs can provide more flexible and agile funds using retroactive or quadratic funding by working together with a consortium of academic, philanthropic, and corporate partners. The scientific data and achievements can be owned and credited using

research NFTs (rNFTs) or intellectual property NFTs (ipNFTs). Peer-to-peer data storage such as Interplanetary File System (IPFS) warrants storing and distributing data enduringly. These cutting-edge crypto-technologies will be able to support a gamut of endeavors in various basic science projects. Thus, I wish to propose establishing gallusDAO to facilitate this chicken cell atlas project in a decentralized manner.

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Table 1 Chicken cell atlas: scRNA-seq data

Focused tissue	Method [a)]	Reference	doi	GEO accession
Embryo	SMART-seq, dissociation, FACS, LCM	Morrison et al. (2015)	10.1002/dvdy.24274	-
Neural crest	SMART-seq, dissociation, FACS	Morrison et al. (2017)	10.7554/eLife.28415	GSE108230
Primitive streak	SMART-seq Fluidigm C1	Vermillion et al. (2018)	10.1016/j.ydbio.2018.04.007	GSE89910
Developing limb	10x, dissociation	Feregrino et al. (2019)	10.1186/s12864-019-5802-2	GSE130439
Gonads	10x, dissociation	Estermann et al. (2020)	10.1016/j.celrep.2020.03.055	GSE143337
Neural crest (hindbrain)	10x, dissociation	Gandhi et al. (2020)	10.7554/eLife.57779	PRJNA624258
Retina	10x, dissociation	Hoang et al. (2020)	10.1126/science.abb8598	https://github.com/jiewwwang/Single-cell-retinal-regeneration
Skeletal muscle and fat (breast)	10x, dissociation	Li et al. (2020)	10.1186/s12864-020-07136-2	CRA002353
Developing retina	10x, dissociation	Tegla et al. (2020)	10.7554/eLife.54279	GSE142244
Limb muscle	10x, dissociation	De Lima et al. (2021)	10.1038/s41467-021-24157-x	GSE166981
Primitive streak	inDrops , dissociation	Guillot et al. (2021)	10.7554/eLife.64819	GSE161905
Cochlea	SMART-seq, dissociation	Janesick et al. (2021)	10.1016/j.celrep.2021.108900	https://umgear.org/dataset_explorer.html?search_terms=33761346

Developing heart	10x, dissociation	Mantri et al. (2021)	10.1038/s41467-021-21892-z	GSE149457
Retina	10x, dissociation	Yamagata et al. (2021)	10.7554/eLife.63907	GSE159107
Pituitary	10x, dissociation	Zhang et al. (2021)	10.3389/fphys.2021.562817	CRA003604
Developing hypothalamus	10x, methanol	Kim et al. (2022)	10.1016/j.celrep.2021.110251	GSE171649
Lymphocyte	10x, suspension	Qu et al. (2022)	10.3389/fmicb.2022.800618	PRJNA687808
Germ cell	10x, dissociation, FACS	Rengaraj et al. (2022)	10.1016/j.csbj.2022.03.040	PRJNA761874
Neural crest	10x, dissociation	Williams et al. (2022)	10.7554/eLife.74464	GSE131688, GSE181577

a) 10x, 10x chromium system from 10x Genomics (Pleasanton, CA, USA). Dissociation indicates enzymatic dissociation.