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Advancing towards a global mammalian gene regulation model through single-cell analysis and synthetic biology

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Abstract

Engineering complex genetic functions in mammalian cells will require predictive models of gene regulation. Since gene expression is stochastic, leading to cell-to-cell heterogeneity, these models depend on single-cell measurements. Here, we summarize recent microscopy and sequencing-based single-cell measurements of transcription and its chromatin-based regulation. Then, we describe synthetic biology methods for manipulating chromatin, and highlight how they could be coupled to single-cell measurements. We discuss theoretical models that connect some chromatin inputs to transcriptional outputs. Finally, we point out the connections between the models that would allow us to integrate them into one global input–output gene regulatory function.

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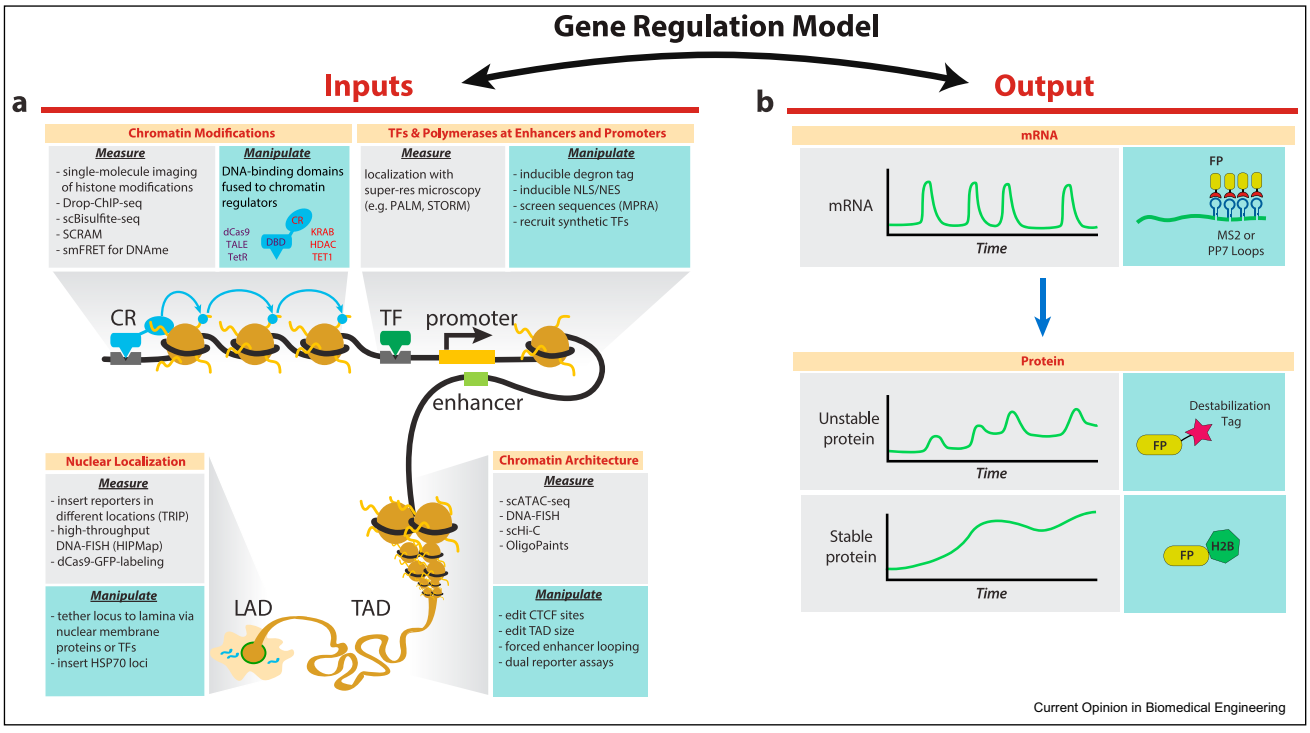
Introduction

Transformative increases in our understanding of gene regulation, along with technologies like sequencing and genome editing, allow us to envision a new era of medicine, in which diagnosis is personalized and therapies are targeted to specific genes, through manipulations of their expression patterns and epigenetic states. One key obstacle to realizing this vision is that we currently lack a predictive, quantitative, and unified theoretical framework for eukaryotic gene regulation. Such a framework would provide an effective "input–output" response for each gene, representing the dependence of its expression level and epigenetic memory on inputs like transcription factor concentrations, specific epigenetic marks, and location within the nucleus. Constructing such a framework is challenging because it has to incorporate many distinct mechanisms of gene regulation.

To experimentally map the effective input–output behavior of a gene, two types of technologies are essential. First, because gene regulation is a highly stochastic process, it can only be understood quantitatively through single-cell measurements. Second, the ability to design synthetic genes and alter natural ones is critical for exploring how sequence, chromatin state, cell context, and specific regulatory factors collectively control gene expression. Mammalian single-cell analysis and synthetic biology are rapidly becoming much more powerful, making this an ideal time to address these problems.

Here we review recent work that has taken promising initial steps towards the kind of quantitative, single-cell gene regulation framework that will enable us to understand, predict, and design gene regulatory systems in mammalian cells. We highlight recent developments in measuring the chromatin inputs (Fig. 1a) and transcriptional output (Fig. 1b) using single-cell methods (Table 1), manipulating those inputs with synthetic biology (Table 2), and modeling the input–output function (Fig. 2). We omit many important factors and mechanisms controlling gene expression including: splicing, non-coding RNAs, post-transcriptional regulation (e.g. miRNA), RNA stability and export, translational and post-translational protein control. However, all of these will ultimately need to be incorporated for a more comprehensive description of gene regulation.

Fig. 1



Input-output function of a unified single-cell model of gene regulation. To develop a complete model of gene regulation, a function that relates inputs like transcription factor binding and chromatin state to transcriptional output is required. **(a)** *Inputs: modulators of transcription, single-cell measurements and synthetic manipulation.* Recently developed tools to measure (gray panels) and manipulate (cyan panels) many determinants of gene regulation (i.e. chromatin modifications, transcription factors and polymerases at enhancers and promoters, chromatin architecture, and nuclear localization). *Abbreviations:* ***scBisulfite-seq****: single cell bisulfite sequencing of DNA methylation;* ***smFRET for DNAme****: single-molecule fluorescence resonance energy transfer measurement of DNA methylation variants;* ***SCRAM***: single-cell restriction analysis of methylation; ***Drop-ChIP-seq****: chromatin immunoprecipitation in droplets followed by sequencing;* ***DBD****: DNA-binding domain;* ***CR****: chromatin regulator;* ***TF****: transcription factor;* ***NLS/NES****: nuclear localization/export signal;* ***MPRA****: massively parallel reporter assay;* ***scATAC-seq****: single cell assay for transposase accessible chromatin followed by sequencing;* ***scHi-C****: single cell high-throughput chromosome conformation capture;* ***DNA-FISH****: DNA fluorescent in situ hybridization;* ***CTCF****: CCCTC-binding factor;* ***TAD:*** *topologically associated domain;* ***LAD****: lamina associated domain;* ***TRIP****: thousands of reporters integrated in parallel.* **(b)** *Output: dynamic measurements of transcription at the single-cell level.* Transcription from a specific locus can be monitored by analyzing fluorescently labeled mRNA over time (top panel) [137,166,167]. The RNA is genetically modified to contain MS2 or PP7 RNA loops that are recognized by cognate RNA-binding proteins fused to fluorescent proteins (FP). Transcription activity can also be inferred from the levels of fluorescently labeled proteins over time (bottom panel). For proteins fused to a destabilization tag, the levels of fluorescence mirror the changes in mRNA. For stable proteins (fused to H2B), the slope of fluorescence intensity (change per unit time) reflects the level of transcription [23,168].

Output: measurements of transcription at the single-cell level

Here, we define the output of gene regulation as the level of mRNA produced, as well as its variability over time and from cell to cell. Work in both cell culture and embryos has revealed that transcription occurs in bursts, alternating between 'on' and 'off' periods of mRNA production (reviewed in Refs. [1–4]). Mammalian mRNA bursting was indirectly inferred by analyzing mRNA distributions in fixed single cells by microscopy using Fluorescence *in Situ* Hybridization (RNA-FISH) [5]. Theoretically, burst-like gene expression processes produce a negative binomial distribution of mRNA numbers, different from the Poisson distribution that would be expected if mRNA were produced at a constant rate. This difference is especially important when the rate of bursting is low, leading to high cell-to-cell variability in the form of a long tail of cells with large numbers of mRNAs [2]). RNA-FISH has been recently modified to measure hundreds to thousands of mRNA species in single cells by using sequential rounds of hybridization and removal of the fluorescent signal, allowing for "barcoding" each mRNA species [6,7]. These types of multiplexed measurements, including single-cell RNA sequencing methods [8,9], can be used to measure the output of all genes that share a common input in single cells. They can be especially powerful when used as an end-point measurement coupled to a time-course recording of the dynamic history of gene expression (movie-FISH) [10,11].

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