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Lighting the shadows: methods that expose nuclear and cytoplasmic gene regulatory control

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Within cells, myriad interconnected processes orchestrate the progression of gene expression from chromatin, to mRNA, and to protein. Assessment of DNA methylation, histone modification, transcript isoform abundance, and the proteome are frequently performed to examine this progression, but do not resolve many intermediary steps in the coordinated regulation of gene expression. Here, we consider single and multiplexed technologies that yield genome-wide assessment of gene and mRNA activity, from transcription factor access to DNA to *de novo* synthesis of protein. An emphasis is placed on methods that can resolve gene regulatory processes in cells of defined identity within multicellular organs at spatial and temporal scales, leading to more effective design of gene regulatory cassettes for biotechnology.

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Introduction

Dynamic regulation may occur at every step in the flow of genetic information from gene, to mRNA, and to protein in eukaryotes. Numerous genome-scale assays of chromatin state and gene activity have accelerated the recognition of coordinated gene regulation. These include the use of chromatin immunopurification-sequencing (ChIP-seq) to define histone modifications and transcription factor (TF) binding-sites, mRNA-seq to monitor polyadenylated mRNAs, and mass spectrometry to quantify proteins. These powerful technologies have advanced functional analyses of regulatory networks but together do not fully capture the dynamics and nuances of gene expression control in multicellular organisms. For example, ChIP-seq does not expose simultaneous interactions between multiple regions of DNA and/or proteins, and RNA-seq does not yield information on the subcellular location or functional state of transcripts. Moreover, alterations in mRNA abundance do not correlate well with protein levels determined by steady-state proteomics [1••]. In multicellular organisms a protein-coding gene active in one cell may be inactive in other cells of the same organ. Here, recent high-resolution technologies that empower intricate dissection of gene regulation from the initiation of transcription to the *de novo* synthesis of a protein in plants are reviewed along with their multiplexing with methods that resolve processes in cells of defined identity of multicellular organs (Table 1).

Chromatin topology, interactions, and accessibility

In the plant cell nucleus, interactions occur between DNA, proteins, and regulatory RNAs within the context of chromatin structure to enable RNA polymerase II (Pol II) to engage and commence pre-mRNA synthesis of a protein-coding gene (Figure 1). Determination of transcriptional initiation is modulated by an array of factors including higher-order chromatin structure, methylation of cytosine nucleotides, covalent modifications of the histones that form the octamer core of each nucleosome, the abundance, activity and interactions of TFs, and the core transcriptional apparatus, along with exposure of *cis*-regulatory DNA sequences (reviewed by [2]).

At the highest order, the three-dimensional organization of chromosomes within the nucleus functions in gene activity [3,4]. Several inventive techniques provide perspective on the location of individual genes with respect to nuclear architecture, such as gene proximity to a nuclear pore or the juxtaposition of chromosomal regions. Fluorescence *in situ* hybridization (FISH) with 'padlock' probes, having termini complementary to a targeted DNA sequence, can detect a specific gene locus within a nucleus. In its recent application to plants, this method demonstrated the repositioning of a gene to the periphery of the nucleus that was concomitant with elevation of the encoded transcript in response to a light signal [5]. At the genome-wide scale, chromatin conformation capture coupled with sequencing (nicknamed Hi-C) enables recognition of inter-chromosomal and intra-chromosomal interactions within nuclei. This approach provided evidence that proximity of distinct chromosomal regions can influence the activity of genes in plants [6,7•].

ChIP-seq is routinely used to identify locations of TF residency, which is coupled with motif searches and other

Table 1

Multi-scale gene regulation assay multiplexing with nucleus-targeted and protein-targeted capture

Technology	Interaction or RNA population monitored	Performed on plants?	Specific cell-type targeted analysis	Representative citations
DNA based				
Hi-C seq	Intra-chromosomal and inter-chromosomal interactions	Yes	Yes[a]: C, N, I	[3,6]
Padlock FISH	Subnuclear DNA localization	Yes	Yes[a]: C, N, I	[5]
ChIP-seq	DNA–Protein	Yes	Yes[a]: C, N, I	
RE-ChIP-seq	DNA–Protein complex	Yes	Yes: C, N, I	[7•,44]
co-ChIP-seq	DNA–Protein–Protein	No	Yes: C, N, I	[8•]
ATAC-seq	Transposase hypersensitive sites (THS)	Yes	Yes[a]: C, N, I	[10•]
scATAC-seq	Single cell ATAC-seq	No	Yes[a]: C, N, I	[12]
Phospho Pol II ChIP-seq	Transcript elongation	Yes	Yes: C, N, I	[17•]
RNA based				
GRO-seq	Nascent RNA	Yes	Yes[a]: C, N, I	[18,19••]
5′ GRO-seq	5′ transcriptions start site (TSS) region of nascent RNA	Yes	Yes: C, N, I	[19••]
nRNA-seq	Nuclear-localized RNA	Yes	Yes[a]: C, N, I	[25••]
PIP-seq	Mapping of nRNA-protein interactions	Yes	Yes[a]: C, N, I	[24••]
RIP-seq	RNA binding protein associated RNA	Yes	Yes: C, N, I	[27,28]
Polysomal mRNA-seq	Polysome-associated mRNA obtained by conventional sedimentation	Yes	Yes: C	[31]
TRAP-seq	Ribosome-associated mRNA obtained by affinity purification of ribosomes	Yes	Yes[a]: C, T	[31,33–37]
Ribo-seq	Ribosome footprint mapping on RNAs	Yes	Yes: C, T	[31,32]
Protein based				
BONCAT	*De novo* protein synthesis	Yes	Yes	[43••]

[a] Method accomplished in plants with one or more methods targeting components of specific cells including nuclei, proteins, or ribosomes. C, FACS, fluorescence-activated cell sorting; I, INTACT, isolation of nuclei tagged in specific cell types; N, FANS, fluorescence-activated nuclei sorting; T, TRAP, translating ribosome affinity purification.

data to pair functional *cis*-elements and TFs to the genes they control. A challenge to concisely defining transcriptional regulatory networks is the disentanglement of the combinatorial roles of TFs, including factors that do and do not directly bind to DNA. Combinatorial indexed-ChIP (co-ChIP) allows for the identification of multiple protein interactors associated with single DNA fragments in a high throughput manner. Two sequential rounds of ChIP with antibodies that recognize specific DNA associated proteins coupled with unique indexing of the DNA fragments in each reaction allows for the identification of segments of chromatin associated with multiple histone marks [8•]. Co-occurring interactions at the plant nuclear pore complex were resolved by pairing restriction enzyme-mediated ChIP-seq (RE-ChIP) with Hi-C [7•]. This uncovered coordinated chromosomal-interactions, chromatin topology, and protein interactions.

Cytosine methylation and covalent modifications of core histones, including, but not limited to methylation, acetylation, and ubiquitination influence chromatin architecture by affecting DNA-nucleosome interactions, which in turn influence exposure of *cis*-elements via chromatin compaction and relaxation. Genome-wide, regions of relaxed chromatin typically lie just upstream of transcription start sites (TSS) and therefore demarcate actively transcribed regions. The methods DNase I hypersensitive-site sequencing (DNase-seq), formaldehyde-assisted isolation of regulatory elements with sequencing (FAIRE-seq), and micrococcal nuclease sensitive-site sequencing (MNase-seq) identify regions of accessible chromatin. Moreover, the fine-mapping of short unexposed regions within these accessibility sites can yield the precise positions occupied by TFs. The assay for transposase-accessible chromatin using sequencing (ATAC-seq) provides similar information [9]. In the ATAC procedure, isolated nuclei are incubated with an engineered recombinant Tn5 'transposome' that inserts sequencing-compatible adapters into non-compacted regions of the chromatin. ATAC-seq has enabled modeling of environmentally-regulated gene networks in field-grown rice [10•] as well as mapping of TF footprints in Arabidopsis [11••]. Adaptation of ATAC-seq to single-cell systems (scATAC-seq) [12] may enable assay of DNA accessibility in individual cells.

To assuage the challenge of defining processes that occur in specific cells of a multicellular organ, most of these chromatin survey methods can be coupled with technologies that enable the targeted capture of nuclei (Table 1). Fluorescence-activated cell sorting (FACS) can be used on transgenic plants with a sub-set of cells defined by a specific promoter driving a fluorescent protein gene (reviewed by [13]). FACS requires the generation of

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