Trends in Plant Science

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Review

Single-Cell Genomics and Epigenomics: Technologies and Applications in Plants

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The development of genomics and epigenomics has allowed rapid advances in our understanding of plant biology. However, conventional bulk analysis dilutes cell-specific information by providing only average information, thereby limiting the resolution of genomic and functional genomic studies. Recent advances in single-cell sequencing technology concerning genomics and epigenomics open new avenues to dissect cell heterogeneity in multiple biological processes. Recent applications of these approaches to plants have provided exciting insights into diverse biological questions. We highlight the methodologies underlying the current techniques of single-cell genomics and epigenomics before covering their recent applications, potential significance, and future perspectives in plant biology.

A Good Time To Resolve Plant Genomics and Epigenomics at the Single-Cell Level

With the development of sequencing technologies, the emergence of omic tools has rapidly changed our views on plant biology. However, the cellular diversity within a tissue or organism is considerably more complex than can be estimated based on **bulk** (see Glossary) analysis which can only yield population-averaged results [1]. Therefore, the conventional consensus from bulk-based omic studies was challenged as sequencing technologies developed toward allowing smaller and smaller samples, eventually allowing single-cell analysis [2]. Characterizing the single-cell genome is of great interest given that each cell experiences a unique series of DNA synthesis and damage repair events. For example, gametes that have undergone meiosis have distinct genomes from one another [3–7], and even different somatic cells show large genomic variations [8–12]. Furthermore, although they contain nearly identical genomes, different cells within multicellular systems perform distinct functions, which can be largely attributed to their diverse **epigenomes**. Recent studies have revealed that the epigenome is an important dimension given that it is known to regulate multiple biological processes, including cell differentiation [13,14], the cell cycle [15], and cell immunity [16], at the single-cell level. Single-cell sequencing technologies have therefore been applied in multiple **animal models** that have demonstrated its power in dissecting the **cell heterogeneity** that underlies many bulk omic features, including genomic variation, DNA methylation, and chromatin accessibility [17,18]. In recent years the technologies of **single-cell genomics and epigenomics** have made remarkable progress in many aspects such as sensitivity and throughput [19]. These technical advances have provided an unparalleled opportunity to profile cell-specific genomic variations and epigenomic features in **plant models**, and hold great promise in answering a diverse range of plant biological questions at the single-cell level.

Single-cell genomics and epigenomics are highly technology-dependent. Recent technical advances in single-cell sequencing made many omic measurements practicable. In the following we discuss the major progress in sequencing-based technologies specifically associated with single-cell genomics and epigenomics, and we refer to the plant models that have been most

Highlights

The rapidly developing fields of single-cell genomics and epigenomics provide powerful approaches to dissect cell heterogeneity in plants.

The sensitivity and specificity of detecting single-nucleotide variations and copy-number variations via single-cell whole-genome amplification technologies have been continuously improving over the past decades. Recent applications highlight their feasibility and utility in plant single-cell genomics.

Recent technical advances in single-cell epigenomics have enabled comprehensive and accurate profiling of diverse epigenomic features. The cell-specific epigenome facilitates the dissection of molecular mechanisms underlying biological processes in plants.

Overcoming current technical issues and integrating multiomic single-cell sequencing data in plants will provide far greater understanding of plant functional genomics.

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studied. Finally, we propose plant-specific opportunities for the further exploitation of these approaches.

Basic Composition of Plant Single-Cell Genomic and Epigenomic Studies

Generally, there are several basic steps in a single-cell sequencing-based study. The first is the preparation of a cell lysate. However, unlike in animal models, the natural cell wall hinders plant cell isolation and lysis, and specialized protocols are therefore needed (Box 1). Once the plant cell lysate has been prepared, single-cell **whole-genome amplification (WGA)** must be conducted, given that currently no sequencing platform is sufficiently sensitive to detect all DNA molecules within a single cell. Single-cell genomic and epigenomic technologies are all based on single-cell WGA, but the latter is more diverse (Figure 1) because of the introduction of sample preprocessing procedures for capturing different epigenomic features, such as bisulfite conversion for DNA methylation [20] and proximity DNA ligation for chromatin conformation [21]. It is worth noting that the quality of the amplification product (such as the DNA quantity and fragment length) and the extent of artificial sequences (such as primer dimers and read chimeras) depend on the single-cell WGA methods used [17], and need to be carefully evaluated alongside the issue of contamination during an experiment because these factors influence library preparation and/or sequencing depth. The sequencing data should first be mapped to the reference genome by sequence alignment software, and then analyzed via bioinformatics approaches for specific experimental purposes, such as the identification of crossover tracts [4] and inferring missing methylation state within a single cell [22].

Single-Cell Genomic Variations

Cell-specific genomic variations are widespread owing to the stochastic introduction of DNA damage and imperfect repair mechanisms [3–12]. This effect can only be fully detected by single-cell whole-genome sequencing where single-cell WGA is essential. In general, single-cell WGA is applied to detect genomic variations that routinely consist of two classes: **single-nucleotide variations (SNVs)** and **copy-number variations (CNVs)**. Methods under development provide an opportunity to dissect cell-specific genomic variations in single plant cells.

Box 1. Cell Isolation Methods for Plant Single-Cell Sequencing

The first and most basic step in single-cell sequencing is cell isolation, and this is a major challenge for plant cells given their cell walls [6]. The most common solution is enzymatic degradation of the cell wall, which is widely used to prepare protoplasts and has been successfully applied to generate single-cell suspensions for cell typing in *Arabidopsis* roots [88–90], rice leaves [91], and rice seedlings [92]. Protoplast preparation is highly efficient for young tissues, but requires long-term incubation for mature tissues, and may thus disturb the original cell type. Another approach is tissue fixation followed by nuclei preparation [85,93]. In this approach the nuclei are crosslinked to preserve the original information during downstream manipulation. For cell types without mature cell walls, such as microspores, no special treatment is required, but the osmotic pressure of the isolation buffer should be optimized [6].

Following removal of the cell wall, two classes of single-cell isolation strategies may be employed: one-by-one and high-throughput. One-by-one methods isolate one cell per tube, finally constructing a sequencing library for each single cell. In this class, manual isolation via micropipette is commonly used, especially in plants, where microdissection is a prerequisite for isolating specific cell types [7]. In this way, each isolation step can be checked microscopically, therefore minimizing the harvesting of multiple cells, but restricting the rate of isolation. In addition, fluorescence-activated cell sorting (FACS) can efficiently isolate individual cells [94], and laser capture microdissection (LCM) preserves the spatial information of isolated cells [95]. Although flow cytometry can efficiently characterize and sort single cells, it is not applicable to cell types that lack specific markers. Furthermore, the one-by-one methods are time-consuming and laborious when the sample size is scaled up to several thousand cells. These problems led to the development of the second class, high-throughput methods, for large-sample single-cell studies. One way is to separate single cells into solid (e.g., microwell plate [96–98]) or liquid (e.g., microfluidic device [99–102]) compartments for cell barcoding. Using this method, single-cell information can be recovered *in silico* from a bulk library. Another approach is to use fixed cells themselves as the compartment, and requires no additional instrumentation [103]. However, although these high-throughput methods achieve efficient isolation and the cost per cell is low, one-by-one methods currently capture more information from each cell.

Glossary

Artifacts: falsely discovered variations introduced during cell lysis and DNA amplification.
Barcoding: a tagging method that employs specific DNA sequences to ensure that reads from the same cell have identical tag sequences.
Bulk: an ensemble of cells, such as organ, tissue, or cell line.
Cell heterogeneity: differences in omic information between single cells from the same bulk which was thought to be homogeneous.
Copy-number variations (CNVs): genomic variations resulting from differences in the number of copies of particular genomic regions, including duplication and deletion.
Epigenome: the linkage between genome and its functional output, including physical modifications and 3D structure of the genomic DNA sequence.
Microfluidics: the technology concerning control and manipulation of small volumes of fluid. Microfluidic devices have multiple biological applications including single-cell compartmentalization and barcoding.
Plant/animal model: the sample source under investigation; for example, apical meristem in plants and cancerous tissue in animals.
Phase: distinguishing paternal and maternal haplotypes from single consensus sequences.
Single-cell genomics and epigenomics: the field concerning analysis of the genome and epigenome in individual cells.
Single-nucleotide variations (SNVs): genomic variations resulting from nucleotide changes at a single position, such as single-nucleotide mutations.
Split-pool: a process in which fixed cells or nuclei are split into different parts for barcoding followed by their pooling together. Several rounds of spilt-pool can enable barcoding of individual cells in a cell population.
Tagmentation: cutting followed by end-tagging of DNA molecules by transposase.
Whole-genome amplification (WGA): polymerase-based amplification of DNA molecules from the whole genome, generating a sufficient quantity for sequencing.

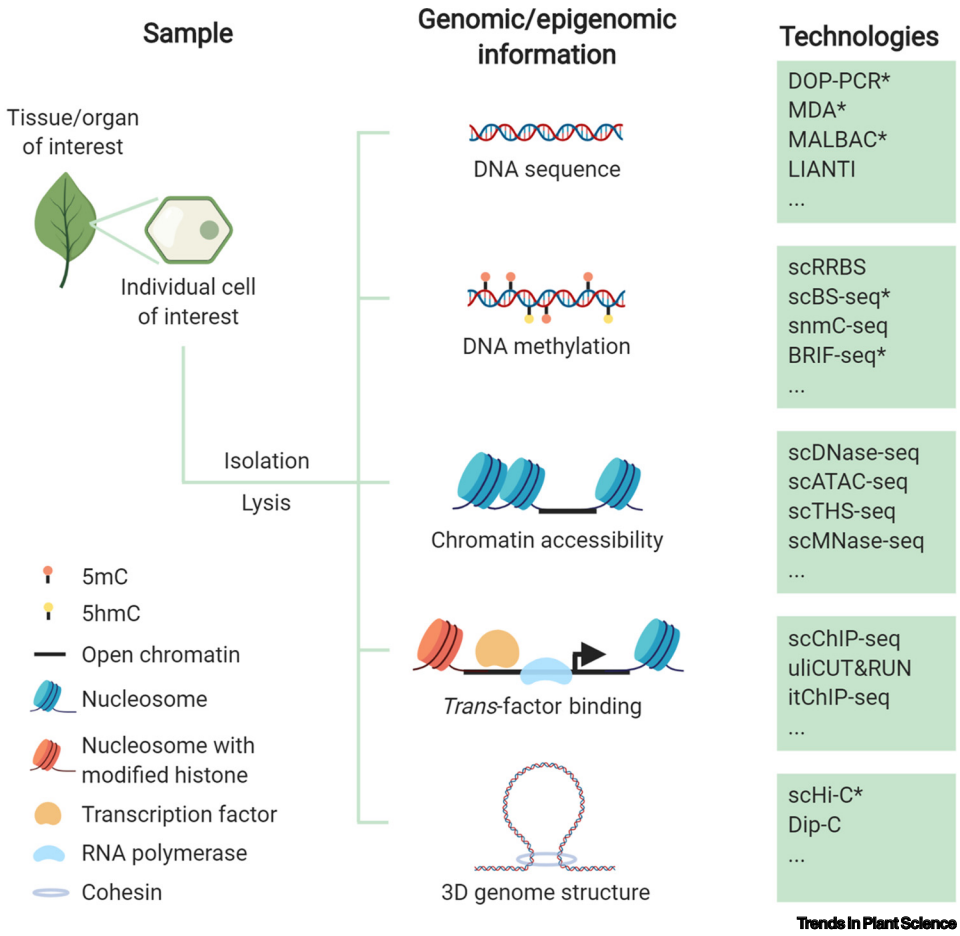

Figure 1. Genomic and Epigenomic Information Can Be Interrogated in a Single Cell via Multiple Single-Cell Sequencing Technologies. A single cell can be isolated in lysis buffer to release DNA molecules for the detection of diverse genomic or epigenomic information by means of different technologies. Technologies already applied in plants are indicated by asterisks. Figure created with BioRender (biorender.com). Abbreviations: 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; BRIF-seq, bisulfite-converted randomly integrated fragments sequencing; DOP-PCR, degenerate oligonucleotide-primed PCR; itCHIP-seq, indexing and tagmentation-based ChIP-seq; LIANTI, linear amplification via transposon insertion; MALBAC, multiple annealing and looping-based amplification cycles; MDA, multiple displacement amplification; scATAC-seq, single-cell assay for transposase-accessible chromatin using sequencing; scBS-seq, single-cell bisulfite sequencing; scCHIP-seq, single-cell chromatin immunoprecipitation followed by sequencing; scDNase-seq, single-cell DNase sequencing; scHi-C, single-cell Hi-C; scMNase-seq, single-cell micrococcal nuclease sequencing; scRRBS, single-cell reduced representation bisulfite sequencing; scTHS-seq, single-cell transposome hypersensitive sites sequencing; snmC-seq, single-nucleus methylcytosine sequencing; uliCUT&RUN, ultra-low input cleavage under targets and release using nuclease.

Single-Cell WGA Techniques

In the past 30 years considerable progress has been made in developing single-cell WGA methodologies. The first attempt involved the use of random primer-based PCR, for example, using degenerate oligonucleotide-primed PCR (DOP-PCR) [23,24]. DOP-PCR usually yields low genomic coverage but features uniform amplification, which is suitable for detecting CNVs but not SNVs. The next single-cell WGA method developed was multiple displacement amplification (MDA) [25,26] which employed isothermal amplification instead of PCR. MDA is especially suitable for SNV analysis because of its high genomic coverage, but is not appropriate for CNV analysis owing to nonuniform amplification. To enable both CNV and SNV analysis, multiple annealing and looping-based amplification cycles (MALBAC) was developed by combining the advantages

of DOP-PCR and MDA to achieve quasi-linear amplification [27]. MALBAC is suitable for both SNV and CNV analysis, but its level of performance is a compromise between DOP-PCR and MDA. More recently, superior single-cell WGA performance has been obtained by **tagmentation**, a technique that uses transposase to simultaneously fragment and tag genomic DNA followed by amplification. The first class of these methods are PCR-based methods, such as direct library preparation (DLP), that display greater uniformity of coverage than any previous methods [28]. However, approximately half of the fragmented template DNA molecules are poorly amplified by PCR because of their complementary end sequences. Multiplex end-tagging amplification (META) overcame this problem by using 20 different tags, leading to only ~5% fragmented template DNA molecules with complementary end sequences, and this largely eliminated the aforementioned inefficiency [29]. The second class relies on *in vitro* transcription rather than on PCR to perform linear amplification, a typical approach being linear amplification via transposon insertion (LIANTI). This method achieved unprecedented sensitivity with respect to SNV and CNV detection [30], and thus holds great potential for future applications.

An obvious limitation of basic single-cell WGA is low throughput. In recent years a major goal in single-cell genomics has been the pursuit of high-throughput methods, in other words multiplexed sequencing of a large number of single cells per experiment. One solution is based on **microfluidic** droplet **barcoding** technologies such as SiC-seq, which enables sequencing of greater than 50 000 single cells per run [31]. Other approaches need no extra instrumentation, and simply use a **split-pool** strategy to construct bulk libraries containing single-cell barcodes. One such approach is single-cell combinatorial indexed sequencing (SCI-seq) in which two rounds of split-pool barcoding were introduced via tagmentation and PCR, respectively [32]. More recently, a high-throughput version of LIANTI, termed sci-L3, has been developed which uses three-round barcoding, thus further increasing both throughput and CNV detection efficiency [33].

SNV-Related Studies

Meiotic recombination is of considerable biological relevance regarding single-cell SNVs concerns because it reshuffles the parental haplotype to generate genetic variations and differs between single gametes. Previous individual-level recombination maps were population-averaged and influenced by potential natural selection [34,35]. However, meiotic crossovers (COs) can be accurately identified in single gametes based on the large amount of SNPs, and thus provides a powerful means to study CO patterns at the single-cell level [4]. In maize, single male [6] and female [7] gametophytes have been isolated and sequenced to generate high-resolution recombination maps, thus providing insights into sex-specific CO patterns in plants. By contrast, single barley pollen genotyping found that segregation distortion in a double-haploid population is not caused by meiosis, strongly indicating that individual-level selection is operating in this population [36,37]. Direct gametophyte sequencing is an ideal approach for plants because of its accuracy and convenience, and we reason that it will become a general route for detecting CO patterns (Figure 2). For example, coupled with genome-editing technologies such as CRISPR/Cas9, it would be feasible to study the influence of CO patterns of specific genetic elements or epigenetic features through high-resolution and/or high-throughput CO detection in single gametophytes. Such an approach would facilitate the accurate validation or identification of functional factors that are associated with CO formation, thereby enabling elucidation of the molecular mechanisms underlying recombination. Moreover, the use of high-resolution single-cell SNV profiling methods would likely enable the detection of gene conversion tracts (the small genomic regions that underwent DNA damage and repair during meiotic recombination [38]) in single gametophytes. Furthermore, by exploiting the haploid nature of gametophytes, heterozygous genomic regions can be efficiently **phased** to separate haplotypes based on parental origin [4,5,39]. The

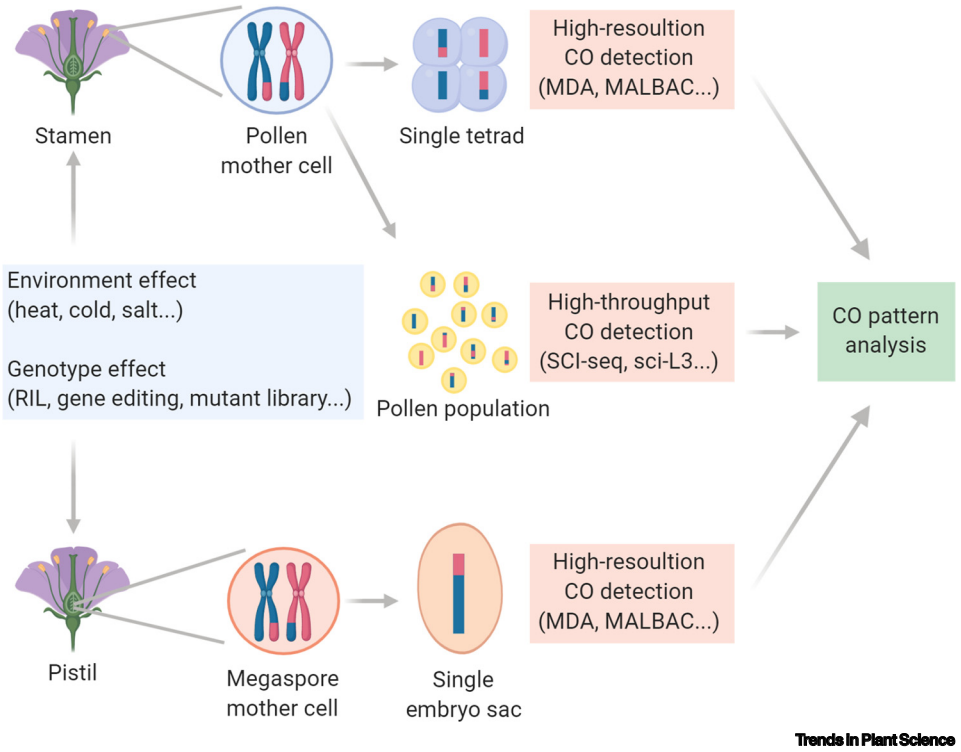

Figure 2. Application of Single-Gametophyte Sequencing for Crossover (CO) Detection. Two strategies are shown to detect COs in single gametophytes: sequencing of single microspores from a tetrad or single embryo sac enables CO location with high resolution; and multiplexed sequencing of a sperm population enables CO location in a large number of single sperms. It is possible to identify factors governing the CO pattern by changing the environment and/or genotype. Figure created with BioRender (biorender.com). Abbreviation: MALBAC, multiple annealing and looping-based amplification cycles; MDA, multiple displacement amplification; RIL, recombinant inbred line; SCI-seq, single-cell combinatorial indexed sequencing.

haplotype information of 12 single-pollen protoplasts was successfully applied to phase the A and B haploid genome of pear, a highly heterozygous fruit tree [40]. Because many plants have highly heterozygous genomes, and many are even polyploid, haploid information of single gametophytes should provide an effective new solution to phase haplotypes for complex genome assembly.

Unlike COs, single-nucleotide mutations are much more difficult to identify in single-cells given the presence of **artifacts** generated by exponential amplification or *in vitro* DNA damage [30,41]. Although linear amplification can reduce artifacts during amplification, *in vitro* DNA damage is difficult to avoid. One way to resolve this problem involves enhancing computational genotyping tools to allow the identification of artifacts. One example is SCcaller, which evaluates the probability of real SNVs by considering allele bias [36]. Similarly, single-cell analysis of SNVs (SCAN-SNV) identifies SNVs by estimating the differences that are likely to occur between amplification bias and common artifacts [42]. A further approach, linked-read analysis (LiRA), captures SNVs phased with somatic SNPs by using heterozygous reads that could carry mate-pair information [9,43]. Another route is through sequencing of kindred cells – progeny cells derived from the division of a single cell [8,30]. This is currently the gold standard for validating single-cell SNVs. Somatic mutations have a great influence on shaping phenotypes and has been widely explored in single cancer cells and single neurons [8–10,44]. Plant shoot meristems can reduce the accumulation of mutations via limited stem cell divisions [45,46], suggesting cell heterogeneity of mutation fixation.

More generally, environment factors such as UV and temperature can alter the somatic mutation frequency in plants [47]. Application of SNV profiling to single plant cells would therefore enhance our understanding of plant-specific DNA damage and repair mechanisms.

CNV-Related Studies

CNVs result from genomic rearrangement, and thereby contribute to cell heterogeneity. Gain or loss of copy number during meiosis is the main cause of aneuploid gamete formation which causes developmental defects in embryos and progeny [17]. Single-gamete sequencing is a sensitive and accurate way to characterize aneuploidy [4,5]. A representative example in plants is single-pollen nucleus sequencing in maize [48]. This work used single-cell CNV analysis of the maize male gametophyte at different developmental stages, and suggested that chromosome fragmentation during post-meiosis mitosis is the leading cause of haploid induction. Interestingly, this study also found that ~9% of pollens in a regular maize line carry aneuploid sperms, a rate much higher than that found in human male sperm [4]. Further studies based on comparisons of CNV patterns in the single gametes of plants and animals at higher resolution would likely better resolve the factors underlying this difference. High-throughput aneuploidy identification could probably be achieved by using high-throughput single-cell CNV profiling methods, which should provide more accurate results because of their larger sample sizes and greater sensitivity, and this has already been demonstrated by their ability to detect rare chromosome mis-segregation events [33]. This method further provided an opportunity to identify elements underlying CNVs in single cells, such as haploid induction genes in plants, by studying genetically different individuals. For plant biology, we anticipate that single-cell CNV profiling methods will largely contribute to basic researches such as studying cell heterogeneity of replication origins [30], as well as practical applications such as evaluating the haploid induction rate.

Single-Cell Epigenomic Information

Although equipped with essentially the same genome, different cell types carry out distinct biological functions that are largely determined by differences in the epigenome, which reflects the functional output of the genome. Based on single-cell WGA, it is feasible to characterize epigenomic features within single cells by means of multiple biochemical approaches. In plants, the epigenome responds to a range of endogenous and exogenous factors, including environmental change [49], biotic signals [50], developmental cues [51], sexual reproduction [52], and plant regeneration [53]. However, the underlying cell heterogeneity in these phenomena is largely unknown. We summarize below major technical advances in profiling single-cell epigenomic features, including DNA methylation, chromatin accessibility, protein–DNA interactions, and 3D genome structure, before highlighting some applications in plant-related fields.

DNA Methylation

DNA methylation (principally 5-methylcytosine, 5mC), which tightly regulates gene expression and cell state, is the best-studied DNA modification [54]. Bisulfite conversion is commonly used to identify 5mC and 5-hydroxymethylcytosine (5hmC) at single-base resolution. Although bulk approaches can be used for single-locus DNA methylation analysis in single cells [55,56], genome-wide analysis required advances in single-cell approaches, including single-cell reduced representation bisulfite sequencing (scRRBS) [57] and single-cell bisulfite sequencing (scBS-seq) [20]. Many aspects of single-cell DNA methylation profiling methods have been refined In recent years. One such improvement is single-nucleus methylcytosine sequencing (snmC-seq), and its improved version snmC-seq2, that optimize the library preparation process to increase mapping rate [58,59]. Bisulfite-converted randomly integrated fragments sequencing (BRIF-seq) uses MDA instead of PCR to amplify converted DNA, thereby increasing genomic coverage [60]. Another improvement is in throughput, for example, single-cell combinatorial indexing for

methylation analysis (sci-MET) incorporates tagmentation and primer extension for two rounds of split-pool barcoding to allow multiplex sequencing of scalable single cells, and also reduces the cost per cell and further increases the mapping rate [61]. Given the substantial DNA loss taking place during bisulfite conversion, a computational approach based on deep neural networks, termed DeepCpG, was developed to predict the missing methylation states in single-cell analysis [22]. DNA demethylation and re-establishment are key processes in rebuilding epigenetic landscapes across generations [52]. Given that each cell undergoes its respective developmental route, single-cell DNA methylation profiling in germ cells and preimplantation embryo deepens our understanding of epigenetic reprogramming [62,63]. In maize, single microspore analysis revealed that the DNA methylation state is similar among the four microspores within a single tetrad, but differs significantly among tetrads, suggesting a novel reprogramming mechanism in plants [60]. We reason that, with the help of the newly developed techniques described above, profiling at single-cell resolution of plant epigenetic states during gametogenesis and embryogenesis will greatly benefit the dissection of plant-specific reprogramming mechanisms (Figure 3).

Protein–DNA Interactions

In addition to DNA modifications, protein–DNA interactions also play important roles in regulating genomic activity. The most common DNA-binding proteins are histones, which package the linear DNA molecule into strings of nucleosomes in eukaryotic cells. The physical accessibility of DNA is mediated by nucleosome sliding that modulates transcription factor (TF) binding and reflects regulatory potential [64]. The development of single-cell nucleosome-occupancy profiling methods in recent years has facilitated the identification of *cis*-elements at the cell type level rather than at the traditional tissue level. Single-cell DNase sequencing (scDNase-seq) captures the DNA released from cell-specific DNase I hypersensitive sites, which represent accessible genomic regions [65]. With the help of transposase, DNA from open chromatin can be cut and tagged simultaneously in a method known as single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) [66]. This has achieved high-throughput in a split-pool manner, termed single-cell combinatorial indexing ATAC-seq (sci-ATAC-seq) [67], and has been applied in

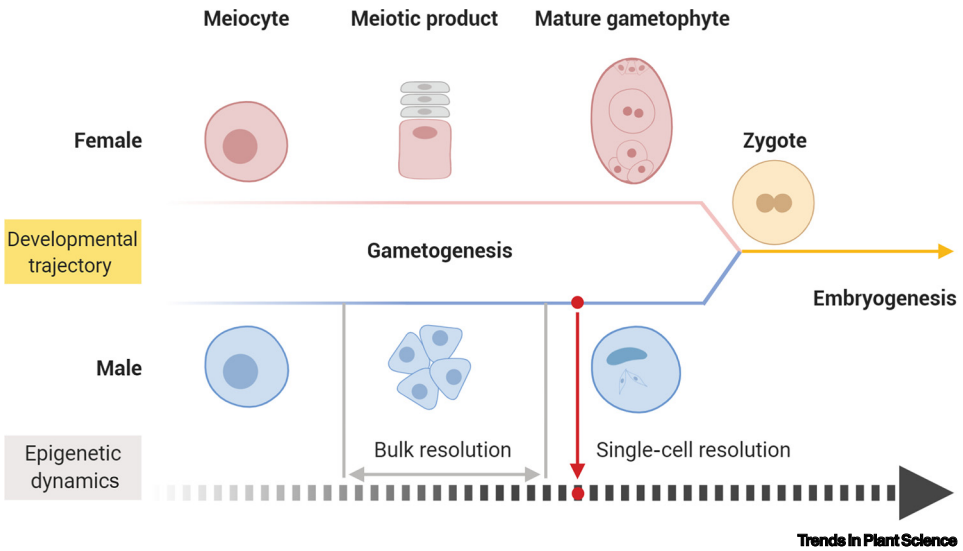

Figure 3. Profiling Epigenetic Dynamics during Gametogenesis and Embryogenesis. Bulk analysis identifies developmental stages based on cell morphology, and thus has poor resolution with respect to the dynamics of epigenetic modifications. Single-cell analysis identifies cell types based on molecular information and preserves single-cell resolution during continuous developmental process. Figure created with BioRender (biorender.com).

profiling chromatin accessibility in greater than 20 000 single nuclei from embryos [68]. Systematic improvement in both amplification and transposase activity was achieved in single-cell transposome hypersensitive sites sequencing (scTHS-seq), which captures more read peaks with a lower sequence bias [69]. An alternative route is to profile nucleosome-binding locations based on micrococcal nuclease which specifically cuts linker sequences between each pair of nucleosomes (single-cell micrococcal nuclease sequencing, scMNase-seq) [70]. Although bulk analysis of chromatin accessibility has been performed in *Arabidopsis* [71], rice [72], and maize [73], it has yet to be achieved at the single-cell level. Applying these methods in different plant tissues and at diverse developmental stages would cast light on cell type-specific *cis*-regulation, thereby providing valuable resources for functional genomic studies.

By contrast to the widely distributed histones, the detection of genomic locations bound by specific *trans*-factors is principally based on ChIP-seq, which generally needs a large number of input cells. Several low-input ChIP-seq methods, including μChIP-seq [74], STAR ChIP-seq [75], and ULI-NChIP [76], have been developed that reduce sample size requirements to as few as several hundred cells; these have been used to profile the landscape of histone H3 lysine 4/lysine 27 trimethylation (H3K4me3/H3K27me3), and lysine 27 acetylation (H3K27ac) in oocytes, sperms and preimplantation embryos. Application of these methods to plant sperm cells, egg cells, zygotes, and early embryos would facilitate the elucidation of plant-specific global remodeling patterns of histone modifications. Compared with the abundant histone modifications, the TF binding landscape is much more difficult to profile [77]. More recently, as few as 100 cells could be used as input to enrich for TF binding sites via ultra-low input cleavage under targets and release using nuclease (uliCUT&RUN), which is based on the micrococcal nuclease-recruited antibody [77]. Another method, termed simultaneous indexing and tagmentation-based ChIP-seq (itChIP-seq), uses transposase to cut the genome before antibody-based purification, enabling the profiling of a transcriptional co-activator using 100 cells, and of RNA polymerase II with 500 cells [78]. These techniques have provided opportunities to obtain the epigenomic landscapes of important DNA-binding proteins from small plant samples. Potential applications of their use include the study of MADS box TFs in early embryo stages [79] and WUSCHEL during shoot apical meristem development [80], holding promise to dramatically increase the resolution of their biological functions in future studies.

3D Genome Structure

DNA/protein modifications, nucleosome positioning, and protein–DNA interactions combine to shape the overall 3D genome structure. A pioneering work optimized the conventional Hi-C protocol to single-cell Hi-C (scHi-C) and proved the feasibility of recovering chromatin conformations within a single cell [21]. Improved versions of this protocol have provided higher resolution and confirmed that the 3D genome structure varies significantly between single cells [15,81,82]. A typical application in plants involved 3D genome reconstruction of single sperm cells, egg cells, and unicellular zygotes in rice, and this revealed the reorganization of global chromatin architectures upon fertilization [83]. However, a current technical limitation of scHi-C is low throughput. To increase throughput, the single-cell combinational index Hi-C (sciHi-C) was developed by using two rounds of split-pool barcoding to enable analysis of several thousand single cells in each assay, which translates to a massively expanded sample size [84]. Conventional scHi-C methods routinely capture tens to hundreds of thousand contacts in a single cell, whereas greater than 10^6 contacts exist in bulk samples, leading to limited resolution. A recent breakthrough, Dip-C, can detect an average of greater than 10^6 contacts in each cell, allowing reconstruction of the 3D genome structure of a single diploid cell [29]. This method makes it possible to interrogate the 3D genome structure of single diploid cells in rice and maize, and even single polyploid cells in wheat and barley, and thus has enormous potential for related studies in our major cereal crops.

Concluding Remarks and Future Perspectives

In the past decade, single-cell sequencing technology has gradually matured to become a powerful tool to dissect biological phenomenon at unparalleled resolution. Plant biology can now enter a new era because single-cell genomics and epigenomics have proved their power in plant researches, including studies of meiotic recombination [6,7,36,37], spermatid chromosome fragmentation [48], epigenetic reprogramming [60], and chromatin conformational reorganization upon fertilization [83]. Application of these techniques to diverse plant models will unveil never before appreciated genomic details in single cells during diverse biological processes throughout vegetative and reproductive growth. We reason that the landscape of plant biology will be revolutionized by ongoing developments in single-cell genomic and epigenomic technologies.

Despite the bright future of single-cell genomics and epigenomics in plants, the present and potential limitations in this field should be carefully evaluated, especially during experimental design. Common technical issues in single-cell sequencing include limited genomic coverage, artifacts, technical noise, and low throughput [1,18,30]. Another plant-specific technical issue concerns initial sample preparation. Given that most single-cell genomic and epigenomic methods have been developed for animal samples, it is worth noting that the experimental procedures must be modified to be compatible with plant samples. This is illustrated by chromatin conformation capture – where the input sample for fixation is intact plant tissues for plant studies but is always cell suspensions for animal studies [15,21,85] – because the preparation of plant cell suspension cultures is highly time-consuming and they are often not fully representative of the cells from which they are derived.

Based on our limited current knowledge, we propose two parallel directions for future developments in this field. The first is to modify current protocols largely created for animal models to render them compatible with plant models, and this will mainly involve reducing cell damage in the sample preparation. Subsequently, it will be necessary to develop new methods to overcome plant-specific issues, such as the low genomic coverage obtained by conventional single-cell DNA methylation profiling in plants with complex genomes [60]. Such a two-step process should enhance both the experimental reproducibility and efficiency of these methods in plant studies. The second direction we propose is to map plant cell atlases based not only on the transcriptome, as discussed previously [86,87], but on the integration of multiomic data including genome, epigenome, transcriptome, proteome, and metabolome, as well as simultaneous multiomic profiling in single cells [19] from multiple plant tissues and developmental stages. In addition, the spatial information of single cells could be recorded in plant cell atlases as the technology moves forward. Such a resource would not only help to extend our understanding of gene function to the single-cell level but also stimulate the development of bioinformatic tools to extract useful information for plant genomics and functional genomics (see Outstanding Questions).

Outstanding Questions

What are the types, and extents, of genomic variations between individual somatic cells from different plant tissues? Do some plant cell types have more intrinsic genomic rearrangements than others, and does this contribute to their biological functions?

Do plant somatic cell types differ in their genomic stability following exposure to homogeneous UV rays? Are the patterns of genomic stability/instability similar to the patterns observed in mammalian somatic cell types?

What epigenetic features determine cell-specific crossover events in meiosis? Which features determine functional megaspore selection?

How are the epigenomic landscapes shaped during plant gametogenesis, oocyte-to-zygote transition, and embryogenesis?

How do *cis*/*trans*-regulatory elements differ between individual cells and cell types in plants?

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