

Nuclear Architecture and Dynamics: Territories, Nuclear Bodies, and Nucleocytoplasmic Trafficking

The nucleus is master of the cellular universe in eukaryotes and home to much of the genome and the machinery for its replication, maintenance, and expression. Although subnuclear compartments such as the nucleolus and Cajal bodies have been known for over a century, studies in the last 2 decades have clearly established that the nucleoplasm is organized into nonmembrane-bound subdomains and bodies. However, detailed understanding of nuclear organization and its dynamics in plants and how they impact DNA replication and gene expression remain to be elucidated. The goal of this Focus Issue is to examine, in the form of Updates, how recent advances contribute to the current understanding of nuclear architecture and dynamics in plant cells and their relevance to nuclear functions and developmental and physiological processes. The Updates also highlight areas that are ripe for mechanistic analysis and technical advances that will enable such studies. We hope that these articles will further stimulate research in this emerging area that has important implications for plant cell and developmental biology and plant responses to the environment.

CHROMOSOME AND CHROMATIN ORGANIZATION AND DYNAMICS

Pawlowski and his colleagues (Tiang et al., 2012) discuss recent advances in chromosome organization and dynamics made using improved fluorescent in situ hybridization protocols as well as live-cell imaging with fluorescent protein (FP)-tagged probes. In particular, they review the concept of chromosome territory in the interphase nucleus and chromosome organization and dynamics in other parts of the cell cycle. They also discuss how the various configurations of chromosome territory observed in different plant species may all achieve the result of bringing telomeric and subtelomeric regions into the vicinity of each other, impacting interchromosomal interactions. They also discuss recent studies that have allowed correlation of cytological phenomena, e.g. chromosome condensation, with molecular events, such as chromatin remodeling, that occur during meiotic prophase. These discussions underscore the progress of chromosome organization studies from descriptive to mechanistic dissection of its functional significance and regulation.

Research on chromatin dynamics dramatically illustrates how nuclear dynamics on the molecular level contributes to gene expression, thereby controlling development. Holec and Berger (2012) compare and

contrast the polycomb group complexes from plants and animals and focus on how chromatin modifications by these complexes confer epigenetic control of coordinated expression of gene networks to mediate major progressions in the plant life cycle. They use deposition, spreading, maintenance, and removal of the histone modification mark (methylation of Lys-27 of histone 3) that accompany the cycle of repression and derepression of the Flowering Locus C to illustrate how various protein and RNA factors mediate chromatin dynamics. They also highlight some major challenges in the field such as understanding the resetting mechanisms for chromatin modification during developmental transitions and how plants make the decision between prolonged maintenance of certain chromatin marks while remodeling others. Rapid advances have been made in the enzymology involved in regulating chromatin dynamics. Liu et al. (2012b) show that transposable element transcriptional reactivation in *Arabidopsis thaliana* is accompanied by an increase in histone acetylation and methylation. They discovered that histone deacetylase6 and DNA methyltransferase physically interact; together, they mediate histone acetylation and modulate DNA methylation status, silencing the transposable element.

NUCLEAR BODIES

The proteins that control many nuclear processes are highly organized into discrete bodies within the nucleus (Misteli and Spector, 2011), which are also referred to as nuclear organelles, enriched in specific proteins and/or RNAs (Mao et al., 2011b). Several different types of nuclear bodies were identified and are thought to function in diverse nuclear activities including gene regulation, processing of different types of RNAs, proteolysis, and DNA replication (Mao et al., 2011b). Four Update articles in this Focus Issue discuss different nuclear bodies and their functions.

Shaw and Brown (2012) provide a succinct overview of the classical and emerging knowledge about the nucleoli, the most prominent of nuclear compartments and the sites for rRNA synthesis, ribosome biogenesis, and a processing and assembly center for many RNA-based processes. They discuss the complexity of nucleolus RNAs and proteins in the context of the emerging understanding of the plurifunctional roles nucleoli play in the cell, including regulating cell cycle, cell death, and stress responses. Shaw and Brown also underscore the view of a dynamic nucleolus with a constant flux of proteins shuttling in and out and reflect on how the constant nucleation and deconstruc-

tion of the nucleolus may relate to some of the cellular processes it regulates. Much remains to be discovered regarding all the nucleolus activities and understanding why these activities are compartmentalized within a defined domain in the nucleus.

Many aspects of plant growth and development from seed germination to flowering are regulated by light. Five classes of photoreceptors sense the light spectrum and elicit appropriate responses, mostly by altering gene expression (Franklin and Quail, 2010; Chen and Chory, 2011). Many of these photoreceptors move to the nucleus in a light-dependent manner and form discrete nuclear bodies called photobodies, which are unique to plants and are regulated by external light cues. The Update by Chen and his colleagues (Van Buskirk et al., 2012) presents recent developments pertinent to composition, dynamics, and potential roles of these bodies in light-regulated gene regulation. It also highlights the utility of forward genetic screens to isolate mutants that are defective in proper localization of photoreceptors to gain further insights into functions of photobodies. They also discuss different potential mechanisms by which photobodies regulate gene expression. A research article by Sokolova et al. (2012) describes how a missense mutation (Ala to Val at position 30) in the N-terminal domain of phytochrome A induces hypersensitivity to continuous low-intensity far-red light and reduces the very low fluency and far-red high irradiance responses. They have shown that this mutation results in reduced affinity to nuclear import facilitators, impairing its nuclear localization and, thereby, altering light responses.

Noncoding small RNAs (micro-RNAs, small interfering RNA, and others) regulate gene expression both at the transcriptional levels by chromatin modifications and/or posttranscriptional level by regulating RNA stability or translation (Czech and Hannon, 2011; Liu et al., 2012a). The update article by Fang and his colleagues (Liu et al., 2012a) provides a nice overview of microRNA (miRNA) biogenesis pathways in plants. They summarize the current status of the composition and function of plant dicing bodies, which are distinct from other nuclear bodies and have been implicated in processing primary miRNA precursors into miRNA. They emphasize that further studies are needed to elucidate all potential roles of dicing bodies in various aspects of small RNA biogenesis and function.

The dependence on constitutive splicing by a cell to generate functional transcripts and the prevalent use of alternative splicing to amplify the capacity of gene functions underscore the need to understand the mechanism of pre-mRNA processing. Reddy et al. (2012) provide an overview of conserved and unique aspects of mRNA processing in plants. Numerous splicing regulatory factors are known to shuttle between the nucleoplasm and the nuclear subdomains called nuclear speckles. Reddy and colleagues provide a systematic discussion of how a variety of recently developed approaches have revealed the temporal and spatial dynamics of these splicing factories.

Developmental and environment signals may all affect the dynamics of the nuclear speckles, such as their sizes and the fluxes of some of the speckle-resident molecules in and out of these compartments. Fundamental aspects regarding these nuclear speckles, such as composition, biogenesis, and functional specialization of subpopulations of these dynamic nuclear structures remain to be elucidated.

NUCLEAR ENVELOPE AND NUCLEOCYTOPLASMIC TRAFFICKING

The nuclear envelope, which separates the nuclear compartment from cytoplasm, is implicated in the organization of chromatin and gene regulation. Nuclear pores regulate the movement of RNA, protein, and RNA-protein complexes into and out of the nucleus. The Update article from Meier and colleagues (Boruc et al., 2012) focuses on recent advances in elucidating the composition and dynamic organization of the nuclear envelopes and nuclear pore complexes. They further define their roles in selective nuclear import and export of macromolecules in plant cells. They also discuss the dynamics of nuclear envelope proteins during cell division and their additional roles in mitosis. Tiang et al. (2012) also discuss the involvement of the nuclear envelope in the formation of telomere bouquet during meiotic prophase.

Most signaling pathways culminate in the nucleus, leading to regulation of expression of specific genes whose products are necessary for eliciting a signal-specific response. Regulated trafficking of proteins, RNAs, RNA-protein complexes, and other molecules into and out of the nucleus is important in diverse processes. The Update by Rivas (2012) covers emerging roles of nuclear trafficking in plant immune responses. She discusses how effectors from diverse pathogens are targeted to the nucleus by coopting the host nuclear import machinery to alter host transcription. She presents an overview of how regulated nuclear localization of pathogen effectors, R proteins, and other host defense signaling proteins modulate plant immunity. Mutations in genes encoding proteins that are part of the nuclear pore complex and nucleocytoplasmic trafficking machinery have been shown to impair plant defense responses, suggesting the importance of dynamic translocation of proteins into the nucleus in regulating the expression of plant defense genes.

New kinds of FPs, called optical highlighters, such as photoactivatable FPs that can be activated to produce fluorescence from a quiescent state or photoconvertible FPs that can be converted from one fluorescent state to the other (e.g. orange to red or green to red), are serving as powerful probes to investigate dynamics of proteins and organelles (Shaner et al., 2007). Monomeric EosFP, a photoconvertible FP, changes its fluorescence irreversibly from green to red when it is exposed to violet-blue light. A research article contributed by Wozny et al. (2012) reports use of monomeric EosFP fused to

a histone to monitor increase in DNA content during endoreduplication based on a shift of red fluorescent nuclei to a green fluorescence state. The use of such optical highlighters (Shaner et al., 2007) offers new avenues to investigate many aspects of plant nuclear architecture and dynamics in live cells.

PERSPECTIVES

Knowledge about the organization of the chromosomes, chromatin, and nuclear bodies in the nucleus and the role such organization plays in nuclear processes is vital to understand how plants grow, develop, and respond to a variety of internal and external cues. As summarized in the articles of this Focus Issue, we are beginning to gain insights into nuclear organization, nuclear compartments and their functions, and the mechanisms that regulate their dynamics in real time. Although targeting signals of some of the resident proteins of plant nuclear bodies are identified (Liu et al., 2012a; Reddy et al., 2012; Van Buskirk et al., 2012), the biogenesis of these subnuclear structures remains largely unknown. Although some clues and experimental approaches may be learned from their animal counterparts (Sharma et al., 2010; Mao et al., 2011a), unique mechanisms supporting the growth and developmental strategies specific to plants are likely to be discovered. The full composition of most plant nuclear bodies is not known. This will require isolation of each type of nuclear body using biochemical and/or microscopic methods followed by proteomic analysis. Also, little is known about the interrelationship(s) between different types of plant nuclear bodies. Simultaneous labeling of multiple nuclear bodies with different FPs fused to proteins that define individual types of nuclear bodies in a single nucleus may provide some clues about this. Precise functions of most of the plant nuclear bodies are still not known. Future studies using genetic, cytological, cell biological, and proteomic approaches and emerging tools are necessary to address these questions. Further elucidation of spatial and temporal organization of nuclear processes and associated factors is necessary to advance understanding of how nuclear architecture and dynamics impact nuclear functions.

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