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Heterochromatin dynamics during developmental transitions in *Arabidopsis* — a focus on ribosomal DNA loci

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ABSTRACT

The *Arabidopsis* chromosomes contain conspicuous heterochromatin domains comprising the repetitive 45S and 5S ribosomal DNA loci as well as centromeric and pericentromeric repeats that organize into chromocenters during interphase. During developmental phase transitions such as seed maturation, germination, seedling growth and flowering that require large-scale reprogramming of gene expression patterns, the organization of repetitive sequences into chromocenters dynamically changes. Here we illustrate recent studies that shed light on the heterochromatin dynamics in cotyledons, the first aerial tissues preformed in the embryo, and in true leaves. We will summarize available data for the 5S rDNA repeat loci, in particular their chromatin organization and expression dynamics during the first days of post-germination development, and discuss how the plant accommodates 5S rRNA transcription during large-scale chromatin reorganization events.

1. Introduction

To ensure successful reproduction, annual plants need to tightly time their major developmental phase transitions such as germination and flowering with environmental stimuli. The switch from a developmental phase to the next requires changes in the spatial and temporal patterns of gene expression. Many signaling cascades and receptors have been described (reviewed in Amasino, 2010; Huijser and Schmid, 2011), as well as specific sets of genes undergoing selective activation or repression through different phase changes (Holdsworth et al., 2008; Schmid et al., 2003). Transcriptional reprogramming of these genes involves active modification of their chromatin structure (Adrian et al., 2009; Exner and Hennig, 2008; He, 2009; Jarillo et al., 2009; Wollmann and Berger, 2012). Interestingly, studies in the model plant *Arabidopsis thaliana* have revealed certain environmental conditions and developmental transitions not only to locally affect chromatin structure of the genes undergoing activation or repression but to profoundly impact higher-order chromatin organization (Mathieu et al., 2003a; Pecinka et al., 2010; Tessadori et al., 2004, 2007a, 2007b; Tittel-Elmer et al., 2010; van Zanten et al., 2011). In a leaf mesophyll nucleus of *Arabidopsis*, two major chromatin states, initially defined by their distinct compaction levels in interphase (Heitz, 1928), can be distinguished: euchromatin, mainly decondensed, contains the majority of genes, and heterochromatin, highly condensed, is composed of silent repetitive sequences and transposable elements. The *Arabidopsis* genome comprises several repeat families including direct centromeric and interspersed pericentromeric repeats that are transcriptionally repressed. Furthermore, the 5S and 45S rDNA repeat arrays that express the ribosomal RNAs, integral components of the ribosome, are partly heterochromatic (Fig. 1A, left). The different heterochromatic repeat loci cluster together into discrete structures, termed chromocenters (Fransz and de Jong, 2011; Fransz et al., 2002) (Fig. 1A), from which gene-rich euchromatin loops emanate thereby building the distinct chromosome territories (Fransz et al., 2002; Pecinka et al., 2004). Cytologically, chromocenters can be revealed as 6–10 intensely 4′,6′-diamidino-2-phenylindole (DAPI)-stained chromatin domains (Fig. 1A, middle) (Dittmer et al., 2007; Fransz et al., 2002), which are highly enriched in DNA methylation and repressive histone modifications such as dimethylation of lysine 9 (K9me2) and monomethylation of lysine 27 (K27me1) of histone 3 (Mathieu et al., 2005; Probst et al., 2003; Soppe et al., 2002). Several studies in the last 10 years have however revealed that this chromatin organization is not static but dynamic and that chromatin undergoes reorganization in certain mutants (Probst et al., 2003; Soppe et al., 2002), specific cell types (e.g. vegetative nucleus of the pollen or the central cell, Slotkin et al., 2009; Ingouff et al., 2010; Pillot et al., 2010) or upon developmental and environmental stimuli in aerial tissues. Indeed, numerous signals, ranging from biotic (e.g. pathogen infections, Pavet et al., 2006) to abiotic (e.g. shade, heat stress, Tessadori

Abbreviations: DAPI, 4′,6′-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization; 5mC, 5 methylcytosine; rDNA/rRNA, ribosomal DNA/RNA; RHF, relative heterochromatin fraction; ROS, repressor of silencing 1; RdDM, RNA-directed DNA methylation; si-RNA, small interfering RNA; TFIIIA, transcription factor IIIA.

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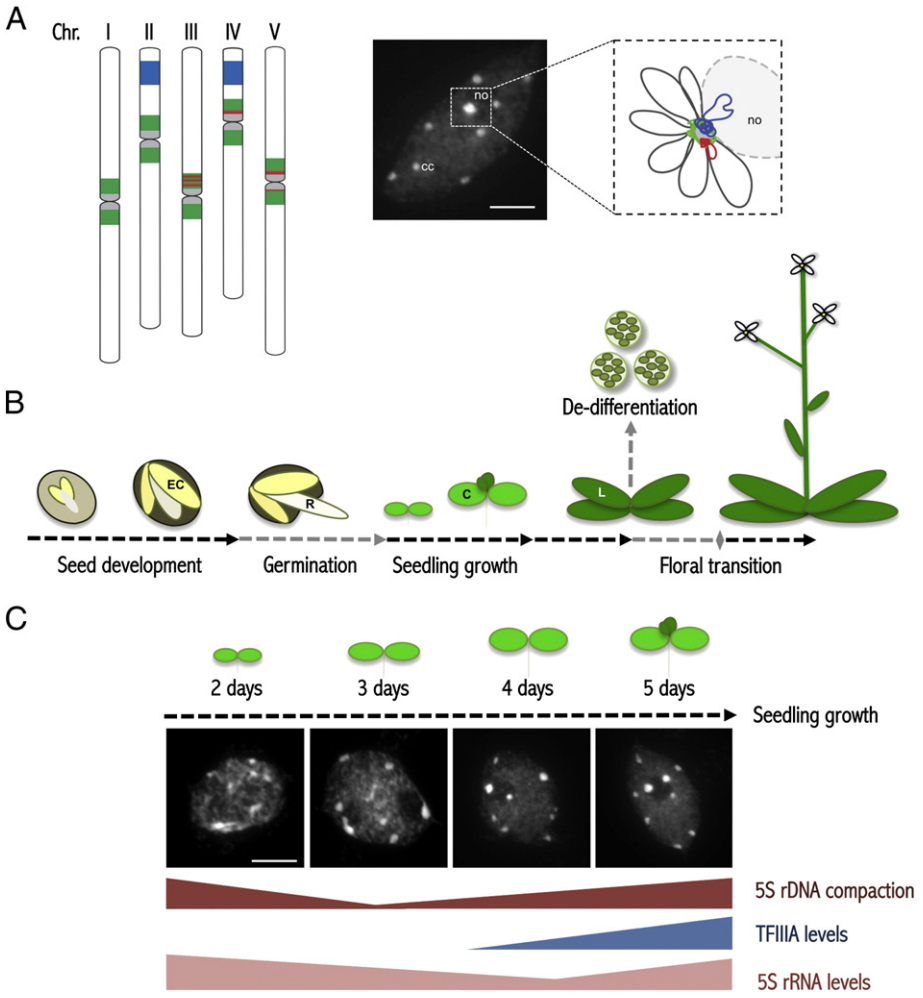

Fig. 1. Chromocenter organization and heterochromatin dynamics during developmental phase transitions. A. Left: Schematic representation of the 5 *Arabidopsis* chromosomes (2n = 10) in the Columbia accession. Chromosome II and IV carry the 45S rDNA loci (blue). The 5S rDNA loci (red) are present on chromosomes III, IV and V, in close proximity to centromeric repeats (180 bp repeats, gray) and inside the pericentromeric domains (green) (AGI, 2000; Fransz et al., 2000; Tabata et al., 2000; Tutois et al., 1999). Middle: Spread of an *Arabidopsis* leaf mesophyll nucleus stained with 4′,6′-diamidino-2-phenylindole (DAPI). Note the euchromatin in light gray and the nine brightly stained chromocenters (cc). The nucleolus (no) appears as DAPI-unstained region. Scale bar represents 5 μm. Right: Model of an *Arabidopsis* chromocenter of chromosome IV adjacent to the nucleolus (no). The chromocenter of chromosome IV comprises the 45S (blue), the 5S (red), centromeric (light gray) and pericentromeric (green) repeats from which euchromatic loops (dark gray) emanate (modified from Fransz et al., 2002). Parts of the ribosomal DNA repeats which are actively transcribed are represented as 45S rDNA sequences (blue) that loop out from the chromocenter into the nucleolus (Probst et al., 2004) and 5S rDNA (red) loops within the euchromatin compartment (Mathieu et al., 2003a) respectively. B. Overview of developmental phase transitions that implicate important chromatin dynamics. Large-scale chromatin dynamics take place in embryonic cotyledons (EC, yellow) during seed development and germination, in cotyledons (C, light green) during seedling growth from 2 to 5 days post-germination and in leaves (L, dark green) upon de-differentiation into protoplasts or during the floral transition. Developmental transitions globally associated with heterochromatin decondensation are marked with gray, those implicating predominantly heterochromatin condensation with black arrows. The radicle (R) is shown in white in the developing and germinating seed. C. DAPI-stained nuclear spreads of cotyledon nuclei at 2, 3, 4 and 5 days after germination. Note the presence of small pre-chromocenters at day 2 and the progressive formation of chromocenters that reach a mature organization at days 4 to 5. Bars below indicate 5S rDNA chromatin compaction (red), presence of functional 5S-specific transcription factor III A (TFIIIA, blue) and amounts of 5S rRNA (pink) during this developmental time window.

et al., 2009; Pecinka et al., 2010; van Zanten et al., 2010) environmental stimuli but also cellular de-differentiation (Tessadori et al., 2007a) or developmental transitions such as seed maturation and germination (van Zanten et al., 2011), seedling growth (Douet et al., 2008; Mathieu et al., 2003a) and floral transition (Tessadori et al., 2007b) have been shown to result in large-scale reorganization of chromatin (Fig. 1B). The chromatin dynamics can affect both euchromatic and heterochromatic regions (reviewed in van Zanten et al., 2012b), but most studies have concentrated on the fate of the heterochromatic sequences as they can be easily visualized using fluorescence *in situ* hybridization (FISH) and their organization into chromocenters monitored by chromocenter size and fluorescence intensity (Soppe et al., 2002; van Zanten et al., 2012b). Upon most phase transitions studied, heterochromatin undergoes decondensation, a change that seems to be of transient nature, as the initial organization of chromocenters is restored in most cases (Tessadori et al., 2007a and reviewed in van Zanten et al., 2012b). However, while certain similarities between the different chromatin reorganization events have been described, their timing can vary from hours to days and each situation appears to reveal its specific characteristics and may implicate different mechanisms depending on developmental context and tissue.

In this review, we will concentrate on the fate of the heterochromatic sequences organized in chromocenters during developmental phase transitions, as those have been investigated in most studies and will allow comparisons to be drawn between the different analyses discussed. We will first illustrate the characteristics of heterochromatin decondensation during floral transition and de-differentiation of leaf cells into protoplasts and then discuss in detail the chromatin dynamics during germination and early post-germination development that take place in the cotyledon. We will particularly emphasize the chromatin dynamics of a specific set of repetitive sequences, the 5S rDNA repeats, during the first days of post-germination growth