term preservation but is also compatible with desiccation, uncovering a new function for this architecture. Whether this mechanism is common to other plant species is still unknown, yet the ‘germination’ of functional amyloids in plants unlocks a new aspect of plant biology and provides a novel system to understand amyloid regulation in higher organisms.

The implications of plant amyloids for the human diet, health, and agriculture remain to be elucidated. However, the fact that vicilin amyloids resist canning and *in vitro* digestibility assays (mimics of gastrointestinal digestion) indicates that humans are exposed to these assemblies (Figure 1F). It is unknown if we can metabolize and absorb nutrients stored in fibrils, what their relationship with the microbiota is, and, more importantly, whether they are involved in seed allergies, since amyloids are more immunogenic and persistent than their monomeric counterparts. Vicilin and similar seed storage proteins are major allergens from pea. Could partial or incomplete digestion of toxic seed amyloids be the primary cause of seed allergies?

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Forum

Chromatin, DNA Replication, and Transcription: Closing the Triangle

Crisanto Gutierrez^{1,*}

Chromatin features are correctly transferred to the daughter strands during genome replication. Recent discoveries demonstrate that replicative DNA polymerases interact physically with histone dimers and tetramers, facilitating histone transfer at the DNA replication fork. This role may explain the transcriptional phenotypic defects of *Arabidopsis* mutants in genes encoding DNA polymerase subunits.

Genomic integrity must be preserved in every cell and in every cell cycle along the thousands of genome replication rounds

that occur during the development of a multicellular organism. Genome replication requires that not only the genetic material, DNA, but also the chromatin, a macromolecular entity formed by the association of DNA with histone proteins into nucleosomes, is correctly duplicated. Therefore, both DNA and histone proteins, together with their post-translational modifications (PTMs), need to be properly transferred to the daughter chromatin fibers. Since the chromatin landscape is a key component of gene regulation, the transferring process of parental histones and their association with new histone proteins is crucial to maintain the memory of transcriptional programs of parental chromatin in the daughter chromatin. This imposes a tremendous challenge since histones incorporated onto newly synthesized DNA are devoid of most PTMs present in the parental copies. Advances in our understanding on how the histone-transferring process occurs have been slow and hard. Recent discoveries using yeast and animal cells models, however, provide important clues of general relevance on how transferring of parental histones is mechanistically linked to the DNA replication machinery. In fact, they may have a direct impact in the plant field to explain and begin to understand the examples of transcriptional dysregulation gathered over the years that occur in a collection of *Arabidopsis* (*Arabidopsis thaliana*) mutants with altered function of DNA replication enzymes [1].

The replisome is a macromolecular complex of several dozens of proteins responsible for replicating the genomic DNA (Figure 1). It consists of the three replicative DNA polymerase holoenzymes (Pol α , Pol δ , and Pol ϵ), each containing several polypeptides and their accessory factors (e.g., RFC and PCNA) and the replicative CMG helicase (formed by Cdc45, the heterohexameric MCM complex, and the heterotetramer GINS complex), which works ahead of the DNA replication fork [2]. Parental nucleosomes

Figure 1. The Replisome and Histone Transfer. Simplified scheme of a DNA replication fork with a focus on parental histone transfer. Each nucleosome contains two copies of each histones H2A, H2B, H3, and H4. Histone H3 can be the canonical form (H3.1) or the variant (H3.3). The CMG helicase separates the two DNA strands ahead of the fork once the nucleosomes are disassembled in H2A-H2B dimers and H3-H4 tetramers. These interact (green arrows) with replicative DNA polymerases (Pol α -primase, Pol δ , Pol ϵ) that help transfer them onto the newly synthesized DNA (in red). RNA primers (small brown boxes) synthesized by the primase are indicated at the 5' end of the new DNA in the lagging strand. Other components of the replisome have been omitted for simplicity. Once the parental histones are transferred they instruct the formation of new nucleosomes in the daughter strands with the help of histone chaperones (not shown). The landscape of post-translational modifications of newly deposited histone is reformed by Polycomb repressor complex 2 (PRC2), an interactor of DNA polymerases, as well as by histone acetylases (HATs), deacetylases (HDACs), and methyltransferases (HMTs). This figure was generated with [BioRender.com](https://www.biorender.com).

are disassembled ahead of the fork into H2A-H2B dimers and H3-H4 tetramers that are transferred to DNA past the fork, where they instruct the formation of new nucleosomes on the daughter DNA strands. In addition, the replisome associates with histone chaperones, such as NAP and CAF-1 complex, that help deposit newly synthesized H2A-H2B histone dimers and H3-H4 tetramers, respectively, onto the newly synthesized DNA to form nucleosomes of the nascent chromatin (Figure 1). The replicative machine also contains histone acetyltransferases (HATs), histone deacetylases (HDACs), and histone methyltransferases (HMTs) that deposit PTMs on the new histones, thus restoring the chromatin landscape after passage of the DNA replication fork [3]. How does DNA replication and chromatin reconstitution on the daughter DNA

strands coordinate with transcriptional regulation past the DNA replication fork?

Mutations in genes encoding various subunits of replicative DNA polymerases in arabidopsis confer puzzling defects in transcriptional control that have been difficult to explain in molecular terms [1]. The DNA primase is responsible for synthesizing the RNA primers that are then extended by the DNA Pol α holoenzyme to form the Okazaki fragments on the lagging DNA strand. The large catalytic subunit of DNA Pol α (POLA1) is encoded by the gene called *INCURVATA2* (*ICU2*). Mutations in this gene are associated with altered expression of genes involved in flower development [e.g., *AGAMOUS* (*AG*)] and flowering control [e.g., *FLOWERING LOCUS T* (*FT*) and *FLOWERING LOCUS*

C (*FLC*)], among others [4]. DNA Pol δ is involved in both lagging and leading strand synthesis and a hypomorphic allele, *gin5*, in the gene encoding its large catalytic subunit (*POLD1*) shows early flowering as a consequence of *FT* upregulation, among others [5]. The third replicative enzyme, DNA Pol ϵ , participates in leading strand synthesis. Mutations in the gene encoding its large catalytic subunit, *POLE1* (also known as *ABO4*, *TIL1*, and *ESD7*), lead to upregulation of *FT* [6,7] as well as defects in heterochromatin, including derepression of the normal transcriptionally silenced state of a variety of transposons. It is worth noting that DNA pol ϵ interacts with components of the Polycomb repressor complex PRC2 [8]. Therefore, mutations in the large catalytic subunits of the three replicative DNA polymerases lead to transcriptional misregulation in both euchromatin and heterochromatin domains. Altered chromatin landscape has been also observed in these mutants, but the molecular mechanism linking defects in the catalytic subunits of DNA polymerases, histone marks, and transcriptional control has been elusive so far.

Recent studies using yeast and mouse embryonic stem cells as models provide key experimental clues to shed light into such mechanism. The use of the eSPAN strategy (for enrichment and sequencing of protein-associated nascent DNA) has paved the way to a significant advance [9]. It is reported that DNA Pol α can preferentially interact with histone H3-H4 tetramers, expanding previous results on the interaction of DNA Pol δ with H3-H4 tetramers [10]. Furthermore, mutations in the catalytic subunit that impair histone binding also prevent correct histone transfer past the fork, leading to misregulation of coding genes and transposons. It has been also shown that the catalytic subunits of DNA polymerases can physically

interact with H2A-H2B histone dimers [9–11]. These results are crucial because they provide a pathway to place parental histone (dimer and tetramers) near the DNA past the fork. This may serve to reconstitute entire nucleosomes, instructing the deposition of histone PTMs to generate a postreplicative chromatin landscape equal to that of prereplicative chromatin (see also later). Thus, defects in parental histone transfer have major consequences in the chromatin landscape, including primarily, nucleosome assembly without participation of parental histones and defects in establishing a correct combinatorial set of histone PTMs (e.g., H3K4me3, H3K27me3, and H3K56ac). Together, these defects affect substantially the transcriptional status of genes in the postreplicative euchromatin as well as of transposons in heterochromatic domains (Figure 1).

The relevance of these findings for plant research in providing a conceptual basis to understand the transcriptional defects of DNA polymerase mutants arises from the significant homology of replisome proteins in plants, yeast, and animals [12]. This occurs not only at the level of major protein domains but even at the amino acid level in those critical for DNA polymerase activities. As a consequence, it is likely that the DNA polymerase–histone interactions demonstrated in yeast and animal cells also occur in plant cells. Therefore, it is conceivable that altered parental histone transfer in *Arabidopsis* DNA polymerase mutants is at the basis of their altered transcriptional silencing of heterochromatin and some euchromatin genes. Mutations in the chaperones responsible for depositing histones onto newly synthesized DNA exhibit relatively limited defects at the

transcriptional level. Thus, the studies discussed here also highlight the key participation of DNA polymerases in histone transfer and reconstitution of the chromatin landscape and transcriptional programs after replication.

The hallmark of genome replication is to produce two identical copies that are transferred to the daughter cells in mitosis. As discussed earlier, the efficient histone transfer by DNA polymerases facilitates reconstitution of postreplicative chromatin bearing the same features present in prereplicative chromatin. However, this step also provides a window of opportunities to make one or both daughter copies different between them in their chromatin features and/or also from the parental one. Therefore, the ability to reprogram chromatin past the fork provides the basis for acquiring new cell fates characterized by specific chromatin landscapes and transcriptional programs.

Although the new findings are crucial to understand better how DNA replication, chromatin reconstitution, and transcriptional regulation are linked, they still leave a key unsolved question: why and how are only some genes or heterochromatin transposons far more sensitive to altered histone transfer? The answer may be related to their spatiotemporal regulatory patterns, their dependence on specific combinations of histone PTMs, and/or the role of histone chaperones responsible for depositing newly synthesized histone onto the transferred histone dimers and tetramers. These and other unknowns can be now tackled within a new conceptual framework that reveals the complex interactions between chromatin dynamics, genome replication, and transcriptional

regulation, a crucial triangle that is beginning to be closed.

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