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Seeds as Emerging Hotspot for Maintenance of Genome Stability

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Summary Seeds represent a plant developmental stage of key agronomic value. Developing seeds undergo a series of contrasting physiological environments, some of which are accompanied with spontaneous and/or programmed DNA damage. Here, we review recent literature illustrating emerging evidence on the importance of maintaining genome stability during the complex life of a seed.

Key words Seed, Genome stability, DNA damage repair, Structural maintenance of chromosome, Chromatin, Chromosome.

Seeds are a vital component of the world's diet and an important source of raw materials for industry (Tanksley and McCouch 1997). The importance of seeds in human nutrition can be best illustrated by the fact that cereal grains contribute to approximately half of the global per capita energy intake (Bewley 1997). Furthermore, seeds are an ideal material for long-term storage of plant genetic stocks and/or their distribution around the world. Therefore, understanding factors influencing seed development, germination, and longevity is relevant for agriculture and germplasm preservation.

Seed development in angiosperms starts with double fertilization. Here, one sperm cell fuses with the egg cell and develops into the embryo, while the second sperm cell fuses with the central cell and will proliferate into the endosperm (Sargant 1900). From this moment on, both tissues follow very distinct pathways, where the embryo represents the next generation, while the endosperm nourishes and protects the embryo, controls its growth, and acts as an inter-ploidy reproductive isolation barrier (Johnston *et al.* 1980, Erilova *et al.* 2009). All major plant parts form in the growing embryo during early seed development. In parallel, the endosperm stimulated by the maternally expressed AGAMOUS-like (AGL) transcription factors rapidly divides and proliferates into a multi-nucleate syncytium. Soon after, AGLs become epigenetically silenced by the activity of maternally expressed Polycomb repressive complex 2, which induces the syncytium to cellularize. This is an important step necessary for further development, in which the embryo absorbs almost the entire endosperm in many dicotyledons. In monocotyledons, the endosperm continues proliferating and forms the largest part of the seed. During ripening, orthodox seeds undergo strong (up to 95%) reduction in water content (dessication), which allows seeds to survive long periods of unfavorable conditions. Under permissive conditions, orthodox seeds imbibe, *i.e.*, increase their water content, restart the cellular machinery, and germinate (Bewley 1997).

Maintenance of chromosome stability and DNA damage repair (DDR) are important the cellular functions, which are necessary for transfer of the high quality genetic information into the offspring. These surveillance systems appeared early during evolution and consist of specialized sensing, signaling, and repair pathways, which are activated in order to optimally eliminate DNA damage and ensure correct assortment of genetic information into daughter cells. Major DDR pathways include base and nucleotide excision repair (BER and NER, respectively), mismatch repair, non-homologous end joining (NHEJ), and homologous recombination (HR). At the chromosome scale, genome stability is ensured by structural maintenance of chromosomes (SMC) complexes, where cohesin (SMC1-SMC3) facilitates sister chromatid cohesion, condensin (SMC2-SMC4) compacts chromosomes, and SMC5/6 complex (SMC5-SMC6) aids DNA replication and DDR (Losada and Hirano 2005, Hirano 2006, Jeppsson *et al.* 2014).

Seeds are regularly exposed to harsh conditions, *e.g.* UV radiation and oxidative stress, which are known to damage macromolecules and reduce seed performance (Britt 1996, Bailly 2004, Waterworth *et al.* 2015). Recently, several genome stability maintenance pathways

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were found to influence seed development and survival. Here we summarize current findings and suggest that protection of genome stability plays an important role in seeds. One fascinating example is the single strand DNA LIGASE1 (LIG1), whose mutants display a strong maternal endosperm developmental phenotype (Andreuzza *et al.* 2010). *LIG1* mutants show DNA hypermethylation at the *cis*-regulatory sequences of the imprinted genes, which are normally DNA de-methylated in the maternal genome during early seed development. This includes the intergenic subtelomeric repeat (ISR) of a key endosperm developmental regulator MEDEA (Li *et al.* 2015). LIG1 was shown to participate in both short and long patch BER in *Arabidopsis* vegetative tissues (Cordoba-Canero *et al.* 2011). Therefore, the most plausible model for explaining its role in gene imprinting is the presence of a non-canonical BER pathway specialized in dissecting DNA methylated cytosines in plants. The primary step is performed by the bifunctional 5-methyldeoxycytosine-specific glycosylase DEMETER (DME), which is expressed in the vegetative cell in pollen and the central cell in ovules (Choi *et al.* 2002, Gong *et al.* 2002). Absence of functional DME leads to a strong seed developmental phenotype. Based on the experiments with a somatically expressed DME homolog, repressor of silencing (ROS1), this family of glycosylases produces abasic sites, which are processed into gaps flanked either by (i) 3′ phospho-unsaturated aldehyde and 5′ phospho group or (ii) 5′ and 3′ phospho-groups, respectively. The 3′ ends of both types of gaps are processed into –OH groups by AP endonuclease APEL1 (type i) or ZDP phosphatase (type ii). This creates a substrate favorable for filling by DNA polymerase and closing the gap by LIG1. Currently, it is unknown whether loss of function of APEL1 and ZDP leads to early seed phenotypes. The biological function of this pathway differs between sexes. In the maternal genome, it activates imprinted genes necessary for endosperm development, while in the paternal genome, it leads to the release of 24 nt small interfering RNAs from repetitive elements, which are thought to migrate into the embryo and silence any potentially active repetitive sequences (Ibarra *et al.* 2012, Baubec *et al.* 2014). Hence, the primary changes induced by non-canonical BER occur in gametogenesis, but are effective only after fertilization.

Within the first hours after pollination, several rounds of nuclear divisions occur in endosperm syncytium. This requires highly dynamic control of chromosome organization as suggested by the *TITAN* (*TTN*) screen in *Arabidopsis* (Liu and Meinke 1998). *TTN* mutants were selected based on large seeds, with poorly developed embryo and non-cellularizing endosperm, which typically aborted at later stages. The key *TTNs* controlling genome stability are *TTN8* and *TTN7*, encoded by SMC1 and SMC3, respectively, corresponding to the core subunits of the cohesin complex, and *TTN3*, which was mapped to a gene of the core subunit of the condensin complex AtCAP-E1 alias SMC2A (Liu *et al.* 2002). Both *TTN7* and *TTN8* mutants showed the severe phenotype with embryos arrested in the preglobular stage and free nuclear endosperm with condensed chromosomes blocked at mitotic prophase. *TTN3* mutants produced large mitotic figures and an excessive number of chromosomes in the endosperm, but the plants were able to produce viable seeds (Liu and Meinke 1998, Liu *et al.* 2002). This is most likely due to the functional redundancy of *TTN3* with the *AtCAP-E2* (*SMC2B*) homolog, as suggested by the embryonic lethality of their double mutant in *Arabidopsis* (Siddiqui *et al.* 2003). In addition, seed developmental phenotypes were found in *AtCAP-C* (alias *SMC4A*) and *AtCAP-D3* mutants (Siddiqui *et al.* 2006, Schubert *et al.* 2013). Recently, the enigmatic

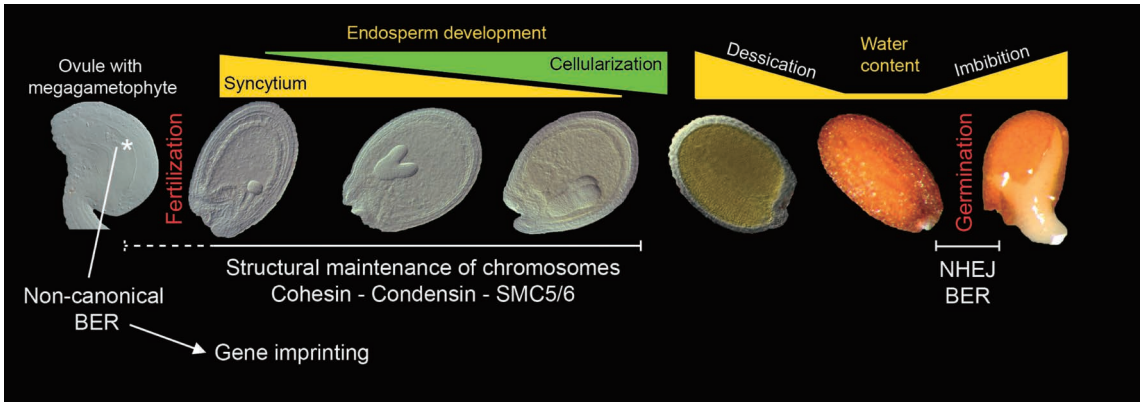

Fig. 1. Overview of genome stability mechanisms during seed development. Images (from left to right) represent an ovule with a mature megagametophyte prior to fertilization and seeds the in globular, heart, torpedo, and mature embryo stages followed by a dry seed and a 24 h imbibed seed of *Arabidopsis thaliana*. During early developmental stages, non-canonical base excision repair (BER) responsible for activation of imprinted genes and structural maintenance of chromosomes (SMC) by all three SMC complexes are active. Wave of reactive oxygen species during imbibition and aging is detoxified mainly by non-homologous end joining (NHEJ) and canonical BER pathways. Individual stages are not displayed to the same scale.

SMC5/6 complex was found to play a role in seed development. Strong mutant alleles of *SMC5*, *SMC6* (as *smc6a* and *smc6b* double mutant), *NSE1*, *NSE3*, and *NSE4A* subunits are embryonically lethal (Watanabe *et al.* 2009, Li *et al.* 2017, Diaz and Pecinka unpublished data). Partially complemented *NSE1* and *NSE3* mutants displayed problems in mitosis, malformation of endosperm nuclei, and embryo arrest at two days after pollination and later seed abortion. Molecular nature of the seed development control by all three SMC complexes remains unknown. Based on the existing data, we conclude that they very likely maintain genome stability in both early stage embryos and the endosperm. Whether and how their functions are connected with other *TTNs* remains to be elucidated.

After embryo expansion, orthodox seeds ripen and desiccate (Roberts 1973, Kranner *et al.* 2010). However, their germination is challenging with respect to genome stability, because of a burst of reactive oxygen species during imbibition (Dandoy *et al.* 1987, Bailly 2004, Waterworth *et al.* 2016). Reactive oxygen species damage DNA by multiple lesions including *e.g.* 8-oxoguanine or DNA double strand breaks (DSB). The major pathway for DSB repair is NHEJ, where ATAXIA-TELANGIECTASIA MUTATED (ATM) kinase signals the presence of DSBs, KU70-KU80 heterodimer stabilizes the broken ends, and DNA ligase 4 (LIG4) connects them. Multiple observations indicate that NHEJ is the dominant DSB repair pathway during imbibition. First, the desiccated quiescent embryo contains cells in the G1-phase, which reduces the chance for homology-based repair *via* HR (Waterworth *et al.* 2015). Second, seeds of LIG4 and KU mutants exposed to genotoxic stress during imbibition germinate later than wild-type seeds, possibly because of a longer time required for repair (Riha *et al.* 2002, Friesner and Britt 2003). However, the opposite phenotype, *i.e.*, faster germination after damage, was observed in ATM mutants (Waterworth *et al.* 2016). This indicates that in the absence ATM, the damage is not detected, which leads to genome instability later during germination. Besides NHEJ, the canonical BER plays role in detoxifying DNA damage in ripened seeds. Two canonical BER DNA glycosylases, formamidopyrimidine-DNA glycosylase1 (FDP) and 8-oxoguanine-DNA glycosylase (OGG), which prefer various substrates in plants (Murphy and George 2005), were found to be up-regulated during seed imbibition in *Medicago truncatula* and *Arabidopsis*, respectively (Macovei *et al.* 2011, Chen *et al.* 2012). In addition, mutations in BER-associated AP endonuclease and ZDP phosphatase caused faster aging during controlled seed deterioration experiments, while OGG overexpression slowed it down (Chen *et al.* 2012, Cordoba-Canero *et al.* 2014). This suggests that BER is necessary for maintaining seed longevity under these simulated conditions.

In conclusion, several stages of seed development are associated with enhanced levels of DNA damage, which can result in reduced seed quality or even loss. Besides already identified factors and pathways, there are other candidates whose molecular mechanism of action and/or corresponding pathways remain unknown, including poly(ADP-ribose) polymerase, plant-specific DNA ligase 6 or several TTNs (Tzafrir *et al.* 2002, Hunt and Gray 2009, Waterworth *et al.* 2010). A full understanding of the role of genome stability in seed development and germination can aid in crop designs with better performance and longer seed shelf life.

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