Cytologia Focus:

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 (Brett 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...
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 glycosylase 1 (FPG) 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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References


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