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Role of AtPolζ, AtRev1 and AtPolη in γ ray-induced mutagenesis

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Although ionizing radiation has been employed as a mutagenic agent in plants, the molecular mechanism(s) of the mutagenesis is poorly understood. AtPolζ, AtRev1 and AtPolη are Arabidopsis translesion synthesis (TLS)-type polymerases involved in UV-induced mutagenesis. To investigate the role of TLS-type DNA polymerases in radiation-induced mutagenesis, we analyzed the mutation frequency in AtPolζ-, AtRev1- or AtPolη-knockout plants *rev3-1*, *rev1-1* and *polh-1*, respectively. The change in mutation frequency in *rev3-1* was negligible, whereas that in *rev1-1* decreased markedly and that in *polh-1* increased slightly compared to wild-type. Abasic (apurinic/apyrimidinic; AP) sites, induced by radiation or generated during DNA repair processes, can pair with any kind of nucleotide on the opposite strand. 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG), induced by radiation following formation of reactive oxygen species, can pair with cytosine or adenine. Therefore, AtRev1 possibly inserts dC opposite an AP site or 8-oxo-dG, which results in G to T transversions.

Key words: ionizing radiation, DNA damage, translesion synthesis, ROS, 8-oxo-dG, Arabidopsis

Abbreviations: UV, ultraviolet; TLS, translesion synthesis; Polζ, DNA polymerase ζ; Polη, DNA polymerase η; GUS, β-glucronidase; ROS, reactive oxygen species; 8-oxo-dG, 7,8-dihydro-8-oxo-2'-deoxyguanosine; 8-oxo-dGTP, 7,8-dihydro-8-oxo-2'-deoxyguanosine 5'-triphosphate

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Ionizing radiation has been applied to various plants for the purpose of generating useful agricultural resources. A variety of ionizing radiation forms, including X rays, γ rays, neutrons and ion-beams, have been used as mutagens for mutation breeding in addition to chemical mutagens.[1] Nevertheless, the molecular mechanism(s) associated with radiation-induced mutations in higher plants remains to be fully understood.

In animals and microorganisms, it is known that a large proportion of mutations occur when damaged DNA is replicated by specific DNA polymerases. This activity is referred to as "translesion synthesis (TLS)," and represents one of the damage-tolerance pathways conserved from bacteria to humans. TLS-type polymerases have a more relaxed active site structure compared to replicases and therefore can act on damaged templates. However, the very flexible nature of the active site can induce high and sometimes fatal, replication errors. In higher plants, the presence of several TLS-type polymerase genes was reported. *AtREV3* encodes the catalytic subunit of AtPolζ.[2] *AtPOLK*, *AtREV1* and *AtPOLH* encode AtPolκ, AtRev1 and AtPolη, respectively.[3-7] In our previous paper, we suggested the role of three TLS-type polymerases, AtPolζ, AtRev1 and AtPolη, in the formation of UV-induced mutations.[8]

Since the variety and ratio of UV-induced DNA damage have been well characterized, and the TLS activity of each polymerase can be examined in vitro, it is relatively easy to speculate on how the TLS polymerases induce mutation following UV-exposure. By contrast, ionizing radiation can induce a variety of damage, including damage to bases and strand breaks, and the role of TLS-type polymerases in radiation-induced mutation is less understood.

In an effort to determine whether TLS polymerases are involved in radiation-induced mutation in higher plants, we analyzed the mutation frequency in Arabidopsis somatic tissues following γ ray irradiation. The reporter gene used for this analysis was the $uidA_{166G\text{-}T}$ gene, which contains a nonsense mutation generated by replacement of the 166th guanine with thymine.[9] The reporter gene

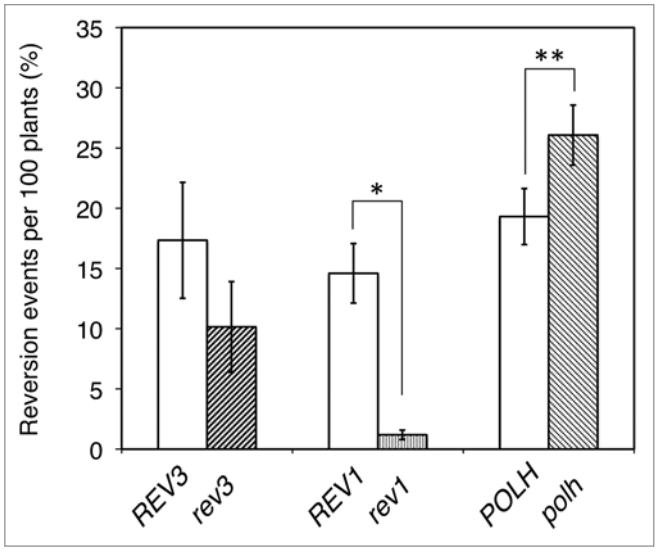

Figure 1. γ ray-induced mutation frequencies in *AtREV3-*, *AtREV1-* and *AtPOLH*-disrupted plants. Wild-type and mutant derived from a single F1 plant were examined concurrently. Bars represent average frequencies per 100 plants derived from multiple experiments. Error bars indicate ±SE. *$p < 0.01$; **$p < 0.05$.

integrated in the Arabidopsis genome will become active when a T-to-G reversion occurs at the 166th thymine. To detect γ ray-induced mutations, transgenic plants carrying the $uidA_{166G\text{-}T}$ were treated with 100 Gy of γ rays and then grown for another 10 days, so that cells with an active *uidA* gene can proliferate and produce a detectable blue sector on somatic tissues.

To investigate the roles of TLS-type polymerases in radiation-induced mutations, we examined the mutation frequencies in disruptants of the *AtREV3*, *AtREV1* and *AtPOLH* genes, *rev3-1*, *rev1-1* and *polh-1,* respectively, and compared these to that of wild-type. The reversion events in *rev3-1* did not change significantly compared to wild-type siblings (**Fig. 1**). This is contrasted with the reduction in UV-induced mutation frequency when AtPolζ is disrupted.[8] However, the reversion events in *rev1-1* plants were less than 1/10 of that in wild-type siblings ($p < 0.01$). This result indicates that AtRev1 plays a role in promoting γ ray-induced mutations. The reversion event in *polh-1* was slightly (~1.4 times) higher than that in wild-type siblings ($p < 0.05$), suggesting that AtPolη plays a role in reducing γ ray-induced mutations.

The frequencies in wild-type, *rev3-1*, *rev1-1* and *polh-1* were 12, 22, 1.9 and 13 times higher, respectively, with γ ray exposure compared to the spontaneous mutation frequency as previously reported.[8] These results indicate that the G to T transversion was greatly induced by γ ray exposure.

Since ionizing radiation can induce a variety of damage to DNA or nucleotide pools, the mechanisms associated with radiation-induced mutagenesis would be more complicated than those pertaining to UV-induced mutagenesis. It is known that some kinds of damage are more abundantly generated by ionizing radiation. Additionally, some kinds of damage are preferentially used as templates or substrates by specific DNA polymerases. Based on previous reports relating to plants or other organisms, we propose two possible mechanisms to account for the γ ray-induced reversion events (**Fig. 2**).

Abasic (apurinic/apyrimidinic; AP) sites represent one of the most abundant DNA lesions that occur spontaneously and are induced by radiation.[10] AP sites can also be generated during the DNA repair process.[11] If the 166th T of our marker gene were lost following irradiation with γ rays, the template would induce various mutations.

Among the TLS-type polymerases, Rev1s share the specific ability to insert dCMP opposite AP sites.[12-14] Therefore, the significant reduction in mutation frequency in AtRev1-knockout plants might be due to loss of dCMP insertion opposite AP sites (**Fig. 2A**). In contrast, it was shown that yeast or human Polηs insert dA or T opposite AP sites or AP-site analogs.[15-17] Thus, the activity of Polη does not seem to contribute toward T to G transversions (**Fig. 2A**). The incidence of mutagenic bypass of AP sites by AtRev1 may be greater when AtPolη is absent, which elevates the mutation frequency slightly.

Given the similar reduction in UV-induced mutation frequencies, we previously suggested that AtRev1 cooperates with AtPolζ to bypass UV-damage.[8] In contrast, no significant change in γ ray-induced mutation frequency was observed in AtPolζ-knockout plants. This suggests that AtRev1 might work independently of AtPolζ when bypassing AP sites, although it is not consistent with previous reports concerning yeast.[15,16]

Radiation damages cells through the formation of reactive oxygen species (ROS). ROS induce oxidative damage of DNA, including strand breaks and base and nucleotide modifications. The formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) represents one of the most abundant and best characterized type of oxidative damage.[18] 8-oxo-dG can pair with cytosine or adenine, inducing frequent base substitutions. In addition to direct oxidation of deoxyguanosine (dG) in DNA, 8-oxo-dG can be generated by the incorporation of oxidized dGTP (8-oxo-dGTP) into DNA during the replication process.[19] 8-oxo-dG in DNA induces mutations when used as a template for the next round of replication. If 8-oxo-dGTP were incorporated in lieu of the 166th T and paired with dC in the next round of replication, it would lead to a T to G transversion (**Fig. 2B**).

It was shown that yeast and human Rev1s insert dC at positions opposite 8-oxo-dG.[13,20] Therefore, the reduction in mutation frequency in AtRev1-knockout plants could be due to loss of dCMP insertion opposite 8-oxo-dG (**Fig. 2B**). Although human and yeast Polηs can insert dC or dA opposite 8-oxo-dG, the insertion efficiencies and dC/dA ratios differ depending on the assay conditions and sequence context.[21-25] Thus, the balance of error-free and error-prone bypass activities of Polη might interfere with the mutation

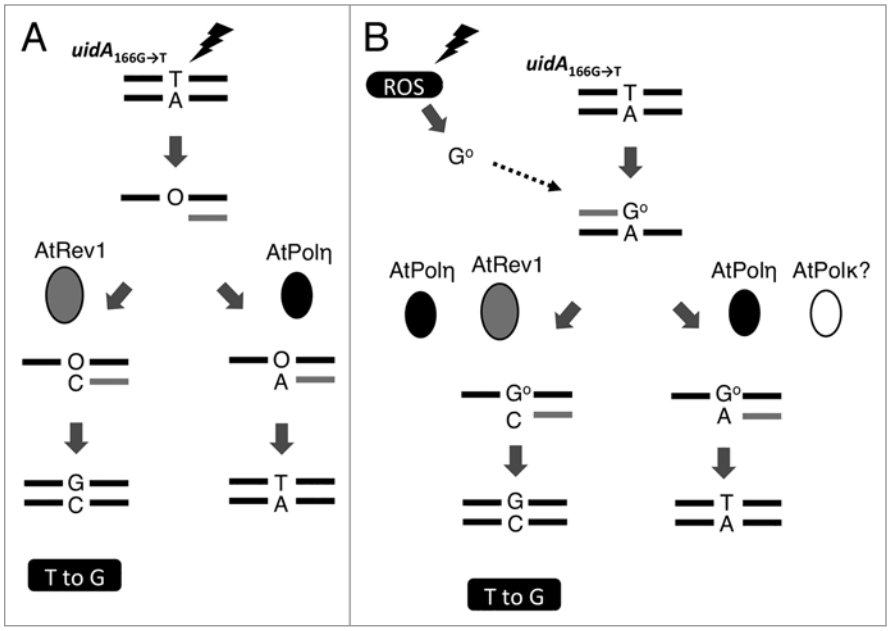

Figure 2. Possible role of TLS polymerases in γ ray-induced mutagenesis. (A) Role of TLS polymerases in the replication of AP sites. Ionizing radiation induces formation of an AP site (O). AtRev1 inserts dC opposite the AP site, leading to a G to T transversion. AtPolη inserts dA or T opposite the AP site, contributing less to G to T transversions. (B) Ionizing radiation induces the formation of reactive oxygen species (ROS) which oxidize guanine (G) in DNA or dGTP, producing 8-oxo-dG or 8-oxo-dGTP (G°). 8-oxo-dGTP is misincorporated opposite adenine (A) through replication. G° is paired with cytosine (C) at the next round of DNA replication, which results in a T to G transversion. AtPolη inserts dC or dA opposite G°, whereas AtRev1 inserts dC opposite G°. Other polymerases including AtPolκ might insert dA opposite G°.

frequency in individual assays. The slight increase in mutation frequency in AtPolη-knockout plants suggests that the ratio of dC insertion by other polymerases was slightly higher when AtPolη is absent.

In yeast, spontaneous mutations in base excision repair (BER)-deficient cells are not reduced by elimination of Polζ, suggesting a minor role of Polζ in 8-oxo-dG induced mutations.[26,27] Our result demonstrating no reduction in mutation frequency in AtPolζ-knockout plants suggests that AtPolζ is also dispensable in terms of 8-oxo-dG induced mutagenesis. However, the root growth of AtPolζ-knockout plants is severely inhibited by γ ray exposure.[2,4] Therefore, it is possible that AtPolζ has other function(s) in radiation-induced damage responses.

In addition to the three polymerases examined in this report, Arabidopsis possesses an additional TLS-type polymerase referred to as AtPolκ. In vitro analysis revealed that AtPolκ preferentially inserts dA opposite 8-oxo-dG,[28] as is the case with human Polκ.[29,30] Therefore, it is conceivable that AtPolκ has a function to promote T to G transversions (**Fig. 2B**). It will be interesting to measure the mutation frequency in AtPolκ-knockout plants following γ ray exposure. Further, analyses of mutation frequencies in BER- or mismatch repair (MMR)-deficient mutants will be necessary to delineate the mechanism(s) of radiation-induced mutagenesis in higher plants.

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