UVR2 ensures transgenerational genome stability under simulated natural UV-B in *Arabidopsis thaliana*

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Ground levels of solar UV-B radiation induce DNA damage. Sessile phototrophic organisms such as vascular plants are recurrently exposed to sunlight and require UV-B photoreception, flavonols shielding, direct reversal of pyrimidine dimers and nucleotide excision repair for resistance against UV-B radiation. However, the frequency of UV-B-induced mutations is unknown in plants. Here we quantify the amount and types of mutations in the offspring of *Arabidopsis thaliana* wild-type and UV-B-hypersensitive mutants exposed to simulated natural UV-B over their entire life cycle. We show that reversal of pyrimidine dimers by UVR2 photolyase is the major mechanism required for sustaining plant genome stability across generations under UV-B. In addition to widespread somatic expression, germline-specific UVR2 activity occurs during late flower development, and is important for ensuring low mutation rates in male and female cell lineages. This allows plants to maintain genome integrity in the germline despite exposure to UV-B.
Plants require sunlight for photosynthesis and developmental regulation. However, ground levels of solar radiation also contain a low proportion of UV-B radiation (UV-B, 280–315 nm), which has multiple effects on plants including photomorphogenic and damaging responses. Photomorphogenic responses are triggered upon UV-B perception by UV-B-Resistance 8 (UVR8) and UV-B-irradiated UVR8 homodimers will monomerize and bind COPII E3 ubiquitin ligase. Reduced COPII activity will allow accumulation of HY5 transcription factor and will trigger UV-B transcriptional response of ~100 target genes and more compact plant growth, including, e.g., reduced plant height and shorter petioles. Furthermore, low UV-B levels boost accumulation of flavonoid pigments, in a TRANSPARENT TESTA 4 (TT4)-dependent manner, which will build up a protective sunscreen layer contributing to UV-B acclimation and even protection against other stresses. Higher natural and, in particular, laboratory-applied UV-B doses cause damage and a burst of reactive oxygen species, damages to cell membranes, UV-B treatments resemble natural conditions during the main A. thaliana-growing season (April/May) along the European north-south UV-Bcline at 60°N, 52°N and 40°N, which can be approximated to Helsinki, Berlin, and Madrid, respectively. Wild-type and all mutant genotypes showed comparable growth at rosette stages under control conditions (Fig. 1b). Under the highest simulated natural UV-B, wild-type and uvr8 plants did not show significantly reduced rosette diameter, while tt4, uvr2, uvr3, uvr2 uvr3 and uvr1 mutant plants did (t-test P values: 5.390E-01, 9.113E-01, 4.3E-06, 1.6E-16, 4.4E-02, 2.6E-16 and 8.2E-03, respectively; Fig. 1b). This suggested that not all A. thaliana mutants found to be UV-B- and/or UV-C-hypersensitive in laboratory would show similar phenotypes under natural UV-B conditions.

Frequency of mutations induced by UV-B treatment. The seeds of control and UV-B-treated plants were grown under non-UV-B conditions and whole genomes of 146 offspring plants, typically five per genotype and treatment, were sequenced (Supplementary Fig. 2 and Supplementary Data 1). This revealed a total of 2,497 novel single-base substitutions and 22 one-to-four base pair deletions. Using di-deoxy sequencing, we confirmed 58 out of 59 randomly selected mutations, suggesting a 1.7% false-positive discovery rate in our analysis (Supplementary Data 2 and Methods). A false-negative mutation discovery rate was estimated to be 0.15% by simulations (see Methods).

Wild-type plants without UV-B treatment accumulated on average 2.6, 2.0 and 2.4 spontaneous mutations per haploid genome and generation (hereafter as 'mutations') in the first (Fig. 1c), the second and the third generations (generation average 2.3), corresponding to 2.2, 1.7 and 2.0 × 10⁻⁸ mutations per site, respectively (Supplementary Data 1). Similar numbers of novel mutations (2.0–5.7) were observed in the progenies of control uvr8, tt4, uvr2, uvr3 and uvr2 uvr3 plants (Fig. 1c and Supplementary Data 1). In contrast, compromised NER in uvr1 plants resulted in 20.3 mutations. This represented 7.8-fold increase (Fisher's exact test, P = 4.9E-12) compared with wild-type and illustrated importance of NER for general genome stability in A. thaliana.

Treatment with 100, 150 and 300 mW m⁻² induced 3.3, 5.0 and 2.8 mutations, respectively, per haploid genome and generation in wild-type plants (Supplementary Fig. 3a). Subsequently, the UV-BBE of 300 mW m⁻² was used as the standard UV-B treatment. Loss of UVR8 and TT4 functions did not significantly change the mutation rates (5.6 versus 7.8 and 5.7 versus 6.7 mutations under control and UV-B; Fisher's exact test P = 0.2203 and 0.6455, respectively; Fig. 1c). In UV-B-treated uvr1 plants, we found 27.4 new mutations, which represented a 1.3-fold increase (Fisher’s exact test, P = 0.03772).

The only drastic increase in mutation rate in a single mutant was observed in the progeny of UV-B-irradiated uvr2 plants containing on average 64.3 new mutations (Fig. 1c). This corresponded to a high 14.7-fold increase over the control uvr2 plants with 4.4 mutations per genome and generation (Fisher’s exact test, P < 2.2E-16). The 7.3 new mutations in UV-B-treated uvr3 plants represented a lower, but still significant 2.1-fold increase over the control treatment (Fisher’s exact test,
UV-B-exposed uvr2 uvr3 double-mutant plants had 66.0 new mutations (Fisher’s exact test, \( P = 2.2 \times 10^{-6} \); Fig. 1c). The progeny of uvr2 uvr3 plants exposed to 0, 100, 150 and 300 mW m\(^{-2}\) UV-B\(_{BE}\) revealed on average 2.0, 39.1, 65.3 and 66.0 mutations per haploid genome and generation, respectively (Supplementary Fig. 3b). This corresponded to 19.5-, 32.6- and 66.0 mutations per haploid genome and generation, respectively.

The UV-B treatment also affected the frequency of non-synonymous amino-acid changes. They were approximately threefold more frequent in UV-B-treated (300 mW m\(^{-2}\) UV-B\(_{BE}\)) uvr2 versus control wild-type plants (14.7% versus 5.9% of all mutations, respectively; Fisher’s exact test \( P = 0.0254 \); Fig. 1d). In absolute terms, this corresponded to 10.2 non-synonymous amino-acid mutations per one uvr2 plant, compared with an average of 0.2, 0.4 and 0.5 such mutations in control wild-type, control uvr2 and UV-B-treated wild-type plants, respectively (Supplementary Table 1). We also found phenotypically distinct plants in the third UV-B-irradiated generation of the double mutant (see example of semidominant mutant in Supplementary Fig. 3c), suggesting an increased functional impact of the mutations induced by the UV-B treatment on gene integrity in UVR2-defective plants.

**Spontaneous and induced mutation spectra in A. thaliana.** To characterize the treatment-specific mutation spectra, we compared mutations from all control plants with those of all UV-B-treated plants with exception of uvr1 samples, which were excluded owing to a 35% rate of A:T→T:A transversions, compared with <10% in the other genotypes (Supplementary Fig. 4a).

Consistent with previous observation of Ossowski et al.\(^{22}\), about half (52%) of all substitutions under UV-B-free conditions were G:C→A:T nucleotide transitions (Fig. 2a). The G:C→A:T frequency increased to 88% after UV-B treatment (Fisher’s exact test \( P < 2.2 \times 10^{-6} \), which led to significantly reduced proportion of all other substitution types (Fig. 2a; Fisher’s exact test \( P < 2.2 \times 10^{-6} \) for control versus UV-B; A:T→G:C, 2.0E-02; A:T→T:A, 9.6E-05; G:C→G:T, 2.1E-05; A:T→C:G, 3.9E-12; G:C→C,G, 1.3E-03). Therefore, simulated natural UV-B caused almost exclusively G:C→A:T nucleotide transitions.
To test whether this holds true in major genome fractions, we quantified mutation spectra in genes and transposons separately (Supplementary Fig. 4b). Under control conditions, G:C→A:T nucleotide transitions remained the major type of change in transposons (66%); however, this trend was absent in genes (23%) where all six possible substitution types showed relatively similar frequencies (10–23%). We also observed more G:C→A:T nucleotide transitions in transposons (65%) than in genes (42%) within the data of Ossowski et al.22 (Supplementary Fig. 4c). Surprisingly, after UV-B treatment, the G:C→A:T transition rate changed and was even larger in genes than in transposons (93% versus 87%; Fisher’s exact test, P value = 0.0038; Supplementary Fig. 4b). Hence, transposons were prone to G:C→A:T transitions under both control and UV-B conditions, while genes only during UV-B treatment.

To find whether spontaneous mutation and those induced by UV-B treatment occurred in a particular sequence context, we performed a motif analysis around mutated sites. This revealed an absence of any specific mutation-prone context in the vicinity of spontaneously mutated G:C→A:T sites in control samples (Fig. 2b). However, within UV-B-treated plants C→T and G→A mutations occurred preferentially within the TC(C/T) and (G/ A)GA contexts, respectively. Such an asymmetric and reverse complementing pattern strongly suggests that: (i) G→A mutations are C→T mutations on the reverse strand; (ii) mutations induced by the UV-B treatment occur predominantly at the 3’ base of the pyrimidine dimer; and (iii) that T(C/T) represents the UV-B-mutation-prone sequence in A. thaliana.

**DNA methylation overlaps with the mutated sites.** On the basis of the preferential UV-B mutagenesis of DNA-methylated cytosines in the CpG context in mammal23,24, we tested for correlation between DNA methylation patterns and mutations induced by the UV-B treatment in A. thaliana. Because DNA methylation is a very stable epigenetic modification, we used existing genome-wide DNA methylation data sets25,26. According to the functional types of DNA methylation in plants25, we classified cytosines in the CG, CHG and CHH sequence contexts (where H is A, T or C) as being either methylated or non-methylated and scored for the methylation status at mutated positions. Stacks’ height indicates the sequence conservation measured in bits44. Symbol of mutated base at the position 0 was size reduced from 2 to 1 bit to reduce graph height. Height of other bases was not changed. Genomes are grouped into control and UV-B samples as described in a.

**Figure 2 | Genomic features of mutated positions.** (a) Proportions of single-nucleotide changes in Ossowski et al.22 control samples (includes all genotypes treated with 0 mWm⁻² UV-BBE; uvh1 was excluded) and UV-B-treated samples (includes all genotypes treated with 100, 150 and 300 mWm⁻² UV-BBE; uvh1 was excluded). Statistical significance in Fisher’s exact test: *P<0.05, **P<0.01, ***P<0.001, n.s. = not significant. (b) DNA sequence motifs associated with control and mutations induced by UV-B treatment. Top images show cytosine and guanine mutation contexts on the forward strand. Bottom images show integrated information from both strands. Stacks’ height indicates the sequence conservation measured in bits44. Symbol of mutated base at the position 0 was size reduced from 2 to 1 bit to reduce graph height. Height of other bases was not changed. Genomes are grouped into control and UV-B samples as described in a. (c) Percentage (x axis) of overlap of mutated positions with DNA methylation, and genome-wide DNA methylation frequencies for cytosines in C, CG, CHG and CHH contexts (where H is A, T or C). Values in columns show absolute number of mutated (Control and UV-B) or genomic positions (Genome) with available DNA methylation information. Statistical significance in Chi-square test with Yates correction: *P<0.05, **P<0.01, ***P<0.001, n.s. = not significant. None of the control versus UV-B comparisons was significantly different (P>0.05). Control samples were grouped as described in a. UV-B containing also 300 mWm⁻² UV-BBE samples. (d) Percentage of mutations in major genome fractions. A. thaliana genome composition according to TAIR8 and TAIR10 annotations. Proportions of spontaneous (Ossowski et al.)19 control sun simulator and UV-B-treatment-induced (300 mWm⁻² UV-BBE) mutations in genes, transposable elements (TE) and intergenic regions. Genomes were as ‘all’ mutations and G:C→A:T mutations only. Individual genotypes were grouped into control and UV-B samples as described in a.
positions. This revealed that both spontaneous and induced mutations overlapped with methyl-cytosines (with the exception of the CHH control group, which contained only 15 testable positions) significantly more often than expected at random based on the genome-wide DNA methylation frequencies (Chi-square test with Yates correction, P values for control versus genome and UV-B versus genome: CNN: 1.12E–04 and <2.2E–16; CG: 1.38E–02 and <2.2E–16; CHG: 6.59E–03 and <2.2E–16; CHH: 6.83E–01 and 3.10E–07; Fig. 2c). Hence, this suggests that methyl-cytosine is prone to mutate under UV-B conditions compared with non-methylated cytosine.

Because DNA methylation is concentrated into transposon-rich chromosomal regions in A. thaliana25,26, we tested whether the mutations show particular genomic distribution. Both control and UV-B treatments led to hypo-accumulation of mutations in genes, relatively random accumulation in intergenic regions and hyper-accumulation in transposons (Fig. 2d). We confirmed this trend using independent data set of Ossowski et al.25 However, UV-B treatment induced ~10% more mutations in genic regions compared with control plants. Therefore, the UV-B treatment adds to the mutagenic effect of DNA methylation, but also affects non-methylated cytosines in genic regions.

Accumulation of induced mutations during development. Early embryonic separation of gametic and somatic cell lineages largely prevents transgenerational inheritance of somatic mutations in mammals27. In contrast, the late separation of germline cells in plants28 allows the inheritance of mutations induced during vegetative growth in cells of the apical meristem into the progeny. Alternatively, mutations can occur later after separation of male and female cell lineages and/or gamete formation. To determine whether mutation induced by UV-B treatment accumulated during particular developmental stages, we analysed the ratio of heterozygous and homozygous mutations in the progeny of the first generation of plants in control and UV-B treatments. If all mutations occurred before the differentiation of the male and female organs, we expected a 2:1 ratio of heterozygous versus homozygous mutations in an inbreeding constitutively monoeocious species such as A. thaliana. We found ratios of 1.4:1 (wild-type control), 2.5:1 (wild-type UV-B-treated) and 1:1 (uvr2 control), but there were significantly 8.1-fold more heterozygous than homozygous mutations (44.22 versus 5.44 per haploid genome, respectively) in the progeny of UV-B-treated uvr2 plants (Fisher’s exact test P values when compared with the other groups: 2.95E–08, 5.83E–05 and 7.97E–05, respectively; Fig. 3a). This strongly suggested that the combination of UV-B treatment with uvr2 genotype leads to mutations mostly after the split of female and male cell lineages. To validate this, we expressed luciferase-tagged UVR2 under control of its native promoter (UVR2promoter::UVR2:LUCIFERASE). The reporter line showed strong UV-B-independent developmentally controlled UVR2 accumulation in meristems (root apical meristem, young leaves, flowers, flower buds, axillary buds, closed anthers and young pistils), scars after petals and sepals and weaker expression in expanded leaves (Fig. 3b–e). The control non-transgenic plants are shown in Supplementary Fig. 5). No expression was observed in green or dry seeds (Fig. 3e). The strong UVR2 expression in floral tissues supported the results of our genetic analysis.

Occurrence of a high number of mutations in male and female cell lineages allowed us to test whether there are sex-specific preferences in mutation accumulation in A. thaliana. We grew uvr2 uvr3 plants under control UV-B-free conditions until bolting, and then exposed half of the plants to UV-B until flowering and subsequently reciprocally crossed UV-B-irradiated and control plants (Fig. 3f). The resulting F1 hybrids were grown under non-UV-B conditions, and genomes of eight plants per crossing

Figure 3 | Developmental aspects of mutagenesis by UV-B treatment. (a) Ratio of heterozygous versus homozygous mutations in UVR2 (wild-type, uvr8, tt4 and uvr3) and uvr2 (uvr2 and uvr2 uvr3) genotypes after one generation of control and UV-B treatment (300 mW m⁻² UV-B86). The 2:1 ratio (horizontal line) was expected if all inherited mutations occurred during somatic development. Mutations above this ratio were likely to originate after separation of male and female cell lineages. *** indicates statistically significant differences to all other samples in Fisher’s exact test, P < 0.001. (b–e) Expression of UVR2-LUCIFERASE translational fusion construct driven by endogenous promoter (UVR2promoter::UVR2:LUCIFERASE). Images on the top/left show plant tissues under white light and those on the bottom/right luciferase signal. All luciferase images were taken using identical exposure time of 1 min, and colour scale at the bottom indicates signal intensity. (b) Ten-day-old in vitro grown plant. Arrowheads indicate luciferase signals in root apical meristems. Scale bar, 5 mm. (c) Leaves dissected from 3-week-old A. thaliana plant organized from the oldest (left) to the youngest (right). Scale bar, 10 mm. (d) Inflorescence. Scale bar, 10 mm. (e) Flower, siliques and seed developmental series. Bottom row, leaf to right: closed flower, flower with emerging pistil, fully opened flower, siliques at different stages and the last opened siliques containing seeds with mature embryos. Hashes: pistils and anthers from (#) opened and (##) closed flowers. Petals and sepals were manually removed. Asterisks: (*) dry and (**) fresh seeds. Scale bar, 10 mm. (f) Genetic test for sex specificity of UV-B-induced mutations. uvr2 uvr3 control and UV-B-irradiated plants (300 mW m⁻² UV-B86) were reciprocally crossed and the number of female- and male-specific mutations was analysed in progeny plants. (g) Boxes show genotype average (middle line), s.d. (left and right margins) and values outside of the s.d. range (horizontal bars) between eight analysed genomes (dots) per experimental point. NS, not significant (Student’s t-test, P = 0.844).
direction were sequenced and analysed. All recovered mutations were heterozygous, excluding self-pollination in any of the 16 analysed genomes (Supplementary Data 2). We found on average 12.4 mutations per UV-B-irradiated mother and 13.3 per UV-B-irradiated father, respectively (nonsignificant in Student's t-test, \( P=0.844 \); Fig. 3g and Supplementary Table 2). This suggests that UVR2 is required for protection of both female and male genome stability, and UV-B treatment induces a similar number of mutations in both sexual lineages.

**Discussion**

Land plants are exposed to solar UV-B during their entire life. In order to minimize UV-B-induced damage, plants use multiple protection and repair pathways, including flavonoid sunscreen, direct reversal of pyrimidine dimers and NER.\(^6\,8,15,29,30\). We determined the frequency of transgenerationally inherited mutations induced by UV-B treatment in *A. thaliana* wild-type and mutant plants treated with simulated solar UV-B, resembling natural conditions from Helsinki (south Scandinavia) to Madrid (central Spain).

The simulated natural UV-B conditions had only a minimal effect on the rosette growth of wild-type Col-0, indicating that they were well in the photomorphogenic range. A wild-type-like phenotype of the UV-B photoreceptor mutant was unexpected as *uvr8* was found to be UV-B-hypersensitive in previous studies\(^19,31,32\). The most likely reasons were acute UV-B stress doses applied to non-acclimated plants and/or use of mutants in more sensitive genetic background in the other studies. In contrast, *tt4* and *uvr2* plants were highly sensitive to the simulated natural UV-B, suggesting that flavonoid production and CPD repair, respectively\(^6,\,\,f3\), are the most important mechanisms sustaining plant growth under simulated natural UV-B.

Under control conditions, we observed on average \(2.3 \times 10^{-8}\) mutations per site, which is approximately threefold more than the previously estimated mutation rates of \(7.1-7.4 \times 10^{-9}\) for *A. thaliana*\(^22,33\). This could be because of presence of UV-A and/or higher photoscopically active radiation (PAR; 400–700 nm; 340 \(\mu mol\,m^{-2}\,s^{-1}\)) fluence rate applied in our control treatment compared with a typical *A. thaliana* growth chamber (100–150 \(\mu mol\,m^{-2}\,s^{-1}\)). However, PAR applied in this study corresponds to a partially shaded natural site, while the full exposure to the sun is simulated using much higher PAR fluence rates (800 \(\mu mol\,m^{-2}\,s^{-1}\) \(f3\)). Simulated natural UV-B conditions caused only small (1.2–2.2-fold) increase in mutation rates of Col-0 wild-type plants. This is in agreement with a previous study, where simulated solar UV-B regimes provoked only one to four germinal somatic homologous recombination events per 250,000 seedlings\(^11\).

The robust protection of *A. thaliana* transgenerational genome stability against UV-B strongly depends on direct reversal by UVR2 CPD photolyase (summarized as schematic model in Fig. 4). The *uvr2* plants accumulated, on average, 64.3 new mutations per haploid genome and generation under the simulated central Spain UV-B regime. Some of these mutations apparently led to a loss of function for housekeeping genes within just three generations. In contrast, loss of UVR3 and UVH1 resulted in a significant, but much lower number of mutations. This may reflect low abundance of UV-B-induced (6–4)PPs (10–25%) relative to CPDs (75–90%) and partial redundancy of NER and UVR3 in repair of (6–4)PPs but not CPDs in *A. thaliana*\(^13,29\).

DNA sequences prone to accumulate UV-B-induced mutations have been unknown in plants. We showed here that sensitivity to our UV-B treatment is determined by both genetic and epigenetic means. Mutations occurred preferentially in the TC dipyrimidine sequence context, and were enriched at methylated cytosines. This differed from spontaneous mutations, which were determined mainly epigenetically by DNA-methylated sites in transposons, but showed no association with particular short sequence motifs. The typical *A. thaliana*-hypermutable sequence TC(T/G) identified here differed from those in humans in at least two aspects. First, we did not observe any CC to TT dinucleotide mutations, which were found frequently in the human eyelid cells\(^34\). Second, in human skin cells the mutated cytosine was frequently followed by a guanine ((T/C)CG)\(^23\). A high proportion of (T/C)CG mutations in humans is most likely caused by the enhanced formation of pyrimidine dimers at methylated cytosines\(^23,24,35,36\), which are found exclusively in the CG context in mammalian somatic cells\(^37\). Absence of such pattern in *A. thaliana* can be explained by presence of DNA methylation in any cytosine context in plants and low number of methylated cytosines in the *A. thaliana* genome\(^25,26\). Although mutations induced by our UV-B treatment were enriched in *A. thaliana* at the positions of methyl-cytosines (27%) relative to genome background (15%), they were not limited to them, and majority of the mutations (73%) appeared at non-methylated positions. This trend was weaker for spontaneous mutations (60% at non-methylated sites) and suggested that UV-B and spontaneous mutations may quantitatively differ in generating C\(\rightarrow\)T transitions via indirect (involving uracil intermediate) or direct conversion, respectively\(^38\).

Animal male and female germline cells separate from somatic cell lineages early during embryo development, and the latter do not divide any more during the post-embryonic phase\(^39\). In contrast, plant germline cells with undifferentiated sex divide several times during vegetative growth and separate into male- and female-specific cell lineages only during late flower development\(^40\). This potentially increases the risk of...
inheriting mutations via somaclonal sectors. In the first post-irradiated generation of control and UV-B-irradiated plants, we found ∼12 ratios of homozygous and heterozygous mutations, respectively. This showed that the spontaneous mutations occurred before the split of male and female cell lineages and the same was true also for mutations induced by UV-B treatment in UVR2 plants. However, there were fourfold more heterozygous mutations in progenies of UV-B-irradiated uvr2 plants. This provided strong genetic evidence that UVR2 prevents UV-B-induced mutations in germline cells mainly after separation of male and female cell lineages, and this UVR2 function seems complementary to its role in resolving CPDs in somatic cells. Induced mutations in germline cells mainly after separation provided strong genetic evidence that UVR2 prevents UV-B-induced mutations in somatic cells. In irradiated generation of control and UV-B-irradiated plants, we inherited mutations via somaclonal sectors. 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Table 1 | Mutation classification thresholds.

| Frequency | Classification                      |
|-----------|------------------------------------|
| >0.9      | Homozygous mutation, accepted      |
| 0.8–0.9   | Undefined, mutation not accepted   |
| 0.3–0.8   | Heterozygous mutation, accepted    |
| 0.1–0.3   | Putative sequencing error, not accepted |
| <0.1      | Reference allele, accepted         |

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Author contributions
A.P., T.P. and K.S. designed the experiments. A.A. and J.B.W. planned and performed sun simulator experiments and determined growth parameters. T.P., A.A. and J.B.W. grew the plants. T.P. prepared sequencing libraries. E.-M.W. analysed whole-genome sequencing data and identified mutations. T.P. and E.-M.W. analysed mutation spectra and associations with genomic features. T.P. cloned the UVR2 reporter construct. A.P. wrote the manuscript with contribution from all authors. All authors read and approved the submitted manuscript.

Additional information

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