Dissecting DNA Damage Responses in Arabidopsis: A High-Throughput Sequencing Approach

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INTRODUCTION

Cells are constantly bombarded with mutagens, both endogenous and exogenous in origin. Endogenous sources of mutation include reactive oxygen species formed during aerobic respiration, replication errors by DNA polymerase, and spontaneous deamination and depurination (Jackson 2009). Exogenous sources of mutation include UV and ionizing radiation, aflatoxins, and polycyclic aromatic hydrocarbons (i.e. compounds found in diesel exhaust and cigarette smoke). As a result, thousands of DNA lesions are created every day. Lesions can stall and impede DNA transcription and replication if they are not removed by DNA repair mechanisms or bypassed by replication.

Phosphoinositide 3-kinase (PI3-K) related protein kinases (PIKKs) regulate the DNA damage response in cells (Cimprich 2008). Commonly referred to as the "sentries to the gate of genome stability," ataxia-telangiectasia mutated (ATM) and ATM and RAD3-related (ATR) promote signaling, cell cycle arrest, and DNA repair in the face of DNA damage.

Arabidopsis thaliana plants lacking ATR, when irradiated with UVB, incur elevated stem cell death in growing root tips (Furukawa 2010). Programmed cell death (PCD) is an important protective mechanism that restores tissue homeostasis in stem cell niches and prevents the accumulation of irreparably damaged cells in tissues, albeit with a delay in growth. Without ATR to stabilize damaged replication forks, double strand breaks (DSBs) occur where there is persistent ssDNA, and ATM or ATR (partially redundant in this signaling capacity) initiate repair or PCD if DSBs accumulate. In plants lacking both ATR and ATM, UV-B irradiation results in less stem cell death than in wild type plants.

In attempts to cross *atr*$^{-/-}$ plants with an EMS-mutagenized line, we discovered a root terminating phenotype which appears to be dependent on ATR and an unknown gene (*ursu*). We are using Illumina high-throughput genome sequencing and bioinformatic techniques to map the location of this additional gene.

METHODS

Next Generation Trait Mapping leverages high throughput DNA sequencing and polymorphisms between cross-fertile varieties to map traits of interest.

1. **Cross mutant with mapping line**
   - Col-0 *atr ursu* X Ler (400,000+ polymorphisms between Col-0 and Ler)
2. **Screen F2 population for trait**
   - Extract DNA, pool, and create Illumina sequencing library from 50-100 individuals
3. **High-throughput sequencing using Illumina HiSeq**
   - ~20X genome coverage of the pooled DNA sample
4. **Bioinformatic analysis**
   - Mapping and Alignment with Short Sequences (MASS) and Next Generation Mapping (NGM) analysis tools (Cuperus et al. 2010 & Li et al. 2008)
5. **Validation**
   - PCR amplification and Sanger sequencing of putative causal mutations identified in the previous step
6. **Further studies to identify gene function**
   - Biochemical and genetic analysis of the identified gene including cloning, complementation, and mutagenesis

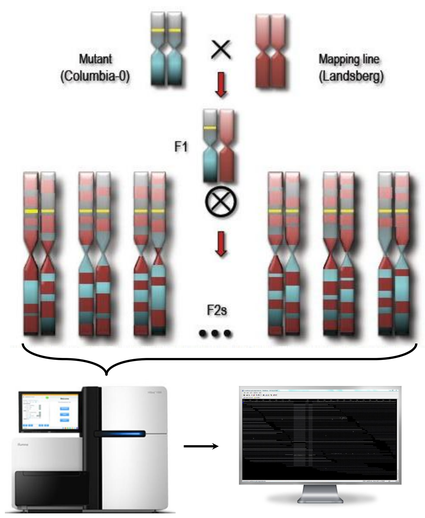

Figure 1. Crossing a recessive mutant with a mapping line to generate a mapping population of F2 individuals. A pooled collection of F2s with the mutant phenotype will possess a random admixture of both mutant and mapping lines except around the mutation of interest, which is detected in sequencing data using bioinformatics tools. The local enrichment of Col-0 specific polymorphism significantly narrows the search for potential causative mutations.

RESULTS

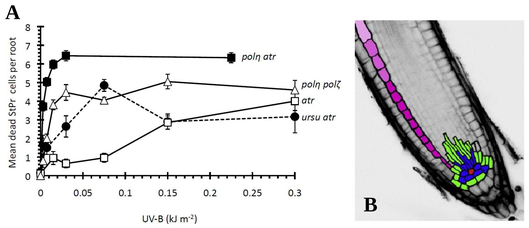

Figure 2. UVB dose-response curves of mutants lacking *polη*, *polζ*, *atr*, or *ursu atr*. (A) Roots of indicated genotypes were irradiated to indicated UVB doses, incubated 24 hours, and 15–20 roots each scored for mean stem and progenitor (StPr) cell death. (B) Arabidopsis root stem cell niche. Stem cells (blue) and their immediate daughters, the progenitor cells (green), divide to give rise to transiently amplifying cells (dark pink) which stop dividing and begin to mature in response to hormone gradients (lighter pinks). The quiescent center is populated by one or two cells (red) (Curtis 2011).

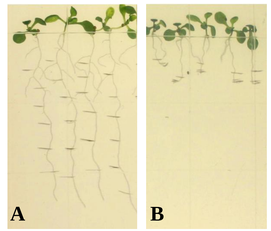

Figure 3. Wild type (A) and *ursu atr* (B) Arabidopsis root tips irradiated with 0.3 kJ m^{-2} UVB. Every 24 hours, beginning 3 days after sowing and continuing through the UVB-irradiation and recovery phases, the back of the plate was marked at the position of each root tip.

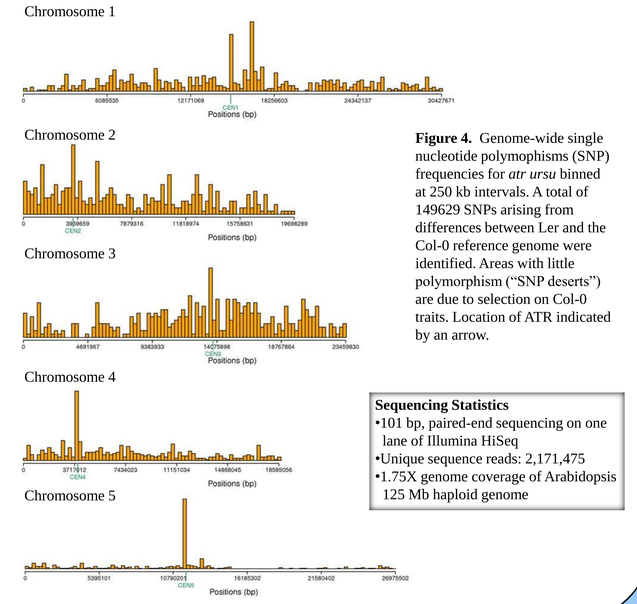

Figure 4. Genome-wide single nucleotide polymophisms (SNP) frequencies for *atr ursu* binned at 250 kb intervals. A total of 149629 SNPs arising from differences between Ler and the Col-0 reference genome were identified. Areas with little polymorphism ("SNP deserts") are due to selection on Col-0 traits. Location of ATR indicated by an arrow.

Sequencing Statistics
- 101 bp, paired-end sequencing on one lane of Illumina HiSeq
- Unique sequence reads: 2,171,475
- 1.75X genome coverage of Arabidopsis 125 Mb haploid genome

SUMMARY

I. Identifying the location and potential mutations in just one lane of Illumina sequencing decreases the time to map a gene from years to months!
II. Low coverage (< 2X) may necessitate repeating the sequencing if other analyses cannot identify a likely candidate for *ursu*.
III. We have identified a few putative gene candidates for *ursu*, and we have confirmed the presence of the mutation using PCR and Sanger sequencing.
   I. Auxin response factor 13 (ARF13), a candidate *ursu* gene, fits in our hypothesis that PCD in the root stem cell niche collapses the auxin gradient. The auxin gradient directs downward growth of the root tip, and its collapse results in growth arrest. Perhaps, if ARF13 does not function, the auxin gradient is not reestablished and growth arrest becomes irreversible.
IV. Parallel analyses to complement and confirm NGM are in progress using different parameters and algorithms (MASS).

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