DNA Rearrangement Mapping Reveals Surprising Patterns of Genomic Instability in Organelles

Samuel Tremblay-Belzile
Director - Dr. Normand Brisson
Université de Montréal

Plant & Animal Genome XXIV
10 January 2016

Plastid genome instability

DNA polymerase: PolIB
Single-strand DNA-binding:
Whirly1 and Whirly3
Recombinase: RecA1

A. thaliana plastid genome

These proteins are encoded in the nuclear genome and targeted to the plastid

Mutant phenotypes

Plastid genome instability

WT polib why1why3 polib

Parent et al. PNAS (2011)

PCR detection of rearrangements

Objective and approach

Analyze the impact of proteins involved in DNA metabolism on organelle genome stability

Develop a next-generation sequencing approach to obtain a genome-wide portrait of DNA rearrangements
**Rearrangement junction sequencing**

- Total DNA → Illumina sequencing
- Alignment on reference genome
- Aligned reads → Unaligned reads (Rearrangements)
- Junction analysis (BLAST)

**Heat map representation**

- Illumina reads (100bp)
- 1st alignment position (kb)
- 2nd alignment position (kb)
- Rearrangements <1000 bp
- Rearrangements ≥1000 bp

**A portrait of plastid genome instability in WT Arabidopsis**

- Proportion of rearrangements (%)
- Del./Dup. vs. Inversion
- Events per 10,000 genomes

**Short-range inversions are a common pattern of instability**

- WT
- 0–49 bp
- 50–99 bp
- 100–249 bp
- 250–1000 bp

**Replication U-turns**

- Original sequence
- Rearrangement
- Replication U-turns have been linked to replication stress

**Portraits of plastid genome instability in Arabidopsis mutants**

- Genome Res. (2015)
Portraits of plastid genome instability in Arabidopsis mutants

Rearrangements < 1 kb

 EVENTS PER 10000 GENOMES

60 times more U-turns in why1why3reca1 mutants than WT

EVENTS PER GENOME

Proposed model

Replication pause

Replication fork collapse

Replication fork restarts on the opposite strand

Accurate replication restart

Short-range inversions are also frequent in Arabidopsis mitochondria

EVENTS PER 10000 GENOMES

Human mitochondria have a larger proportion of short-range inversions

EVENTS PER 100 GENOMES

Ara. mito.

Brain mito.

Liver mito.
U-turns are a major source of instability

Proportion of U-turn-like rearrangements (%)

Genome Res. (2015)

Conclusions and perspectives

1) Short-range rearrangements in organelles have mostly been underestimated in previous studies

2) The abundance of U-turns in all organisms suggest they may play a role in some diseases involving mitochondrial dysfunction (Parkinson, some cancers and neurodegenerative disorders)

3) As sequencing technology improves, this approach could be applied to nuclear genomes

Acknowledgements

Eric Zampini
Étienne Lepage
Sébastien Truche
Dr. Normand Britson

Rearrangement types

No microhomology

Original sequence
Rearrangement

Microhomology

Original sequence
Rearrangement

Detailed method

Replication stress in mutants
U-turn directionality

Upstream

Same Base

Downstream

Legend:
- Red: Upstream
- Black: Same Base
- Green: Downstream

Graph:
- X-axis: Month
- Y-axis: Number of occurrences

Legend:
- June
- July
- August
- September
- October

1/19/2016