Is increased mobility of mobile DNA elements in old cells caused by pH regulation?

Danielle McFee
Department of Biology, Siena College, Loudonville, NY

Introduction

The mechanisms of aging are not completely understood but many factors are associated aging. Retrotransposons are mobile DNA elements that may contribute to aging based on studies conducted in several species. In yeast the Ty1 retrotransposon is more active in older cells compared to younger ones. Recent studies have shown that there is an asymmetry in the cytoplasmic pH between young daughter cells and older mother cells. As mother cells age, their cytoplasmic pH increases, and this difference has been attributed to the activity of the Pma1 proton pump. Pma1 is a cell membrane protein that pumps protons from the cytoplasm into the extracellular space, effectively increasing cytoplasmic pH. The observed asymmetry in pH between mothers and daughters also correlates with the increased Ty1 mobility in mother cells compared to daughters. This correlation could indicate that pH change contributes to the regulation of Ty1 retrotransposition.

Research Question

Does Pma1 proton pump activity impact the retromobility of the Ty1 retrotransposon in yeast?

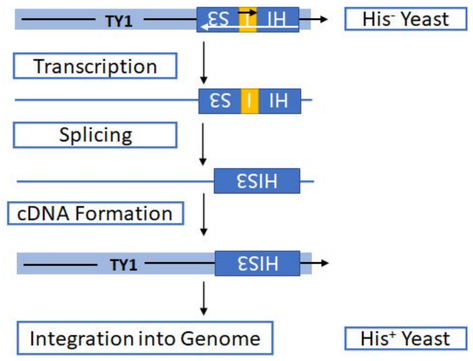

Figure 2. Ty1*his3AI* Assay for measuring Ty1 mobility.

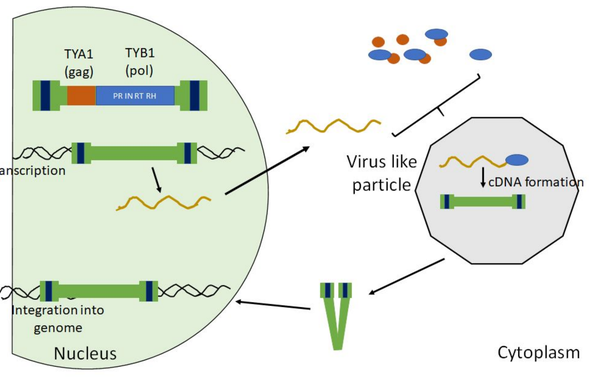

Figure 1. *Saccharomyces* Ty1 retrotransposition cycle. Ty1 is transcribed into RNA and then the RNA is translated to make gag and pol proteins which are used in virus like particle formation (VLP). In the VLP, the Ty1 RNA is reverse transcribed into cDNA which can then be integrated into the genome.

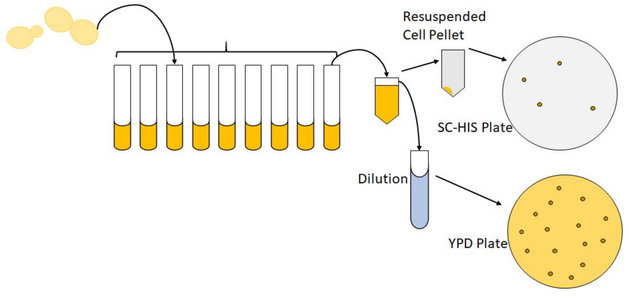

Figure 3. Assay for Ty1 mobility rate. Cells were grown for 2-3 days in YPD broth. Cells spread on YPD were diluted 1000 fold and 2 µL was spread to determine culture density. Remaining cells were spread on SC-HIS plates after resuspension of pellet in water to assess the mobility rate of Ty1. Only cells that have gone through the retrotransposition process will grow on SC-HIS, the number of cells on SC-HIS and culture density calculated based on the YPD are used to calculate the mobility rate.

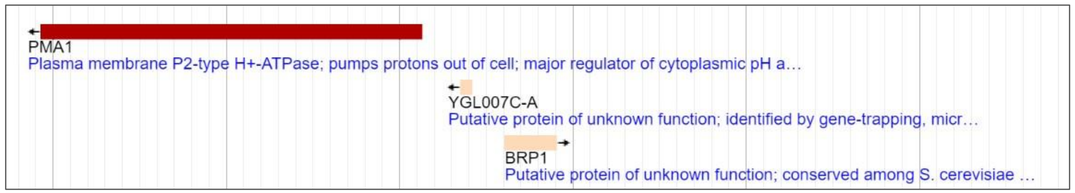

Figure 4. The *PMA1 BRP1* gene region. In *pma1* mutant strains, the *BRP1* sequence in the *PMA1* promoter region has been replaced with *URA3* to decrease expression of the *PMA1* gene and thus the Pma1 proton pump expression. Each column represents 120 base pairs.

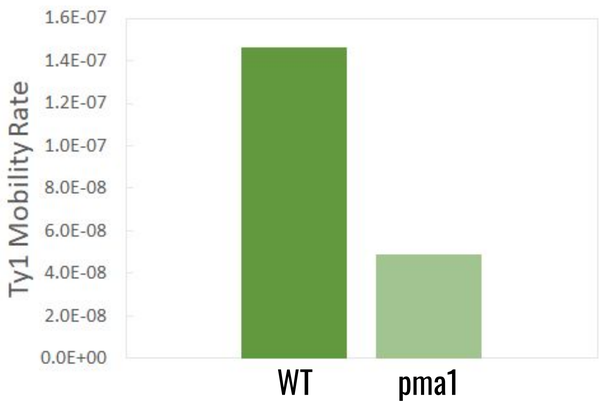

Figure 5. Retrotransposition rate decreased in strain with decreased Pma1 expression compared to wild type Pma1 expression. The *URA3* gene replaced *BRP1* in the mutant strain.

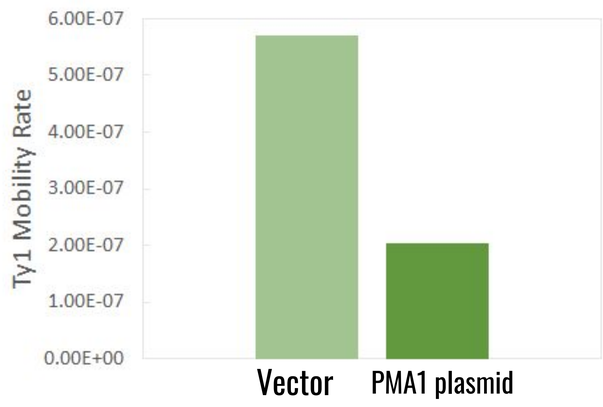

Figure 6. Introduction of *PMA1* plasmid decreased Ty1 mobility rate compared to cells with only vector introduced. Cells with low-copy *PMA1* plasmids were used to determine if over expression of *PMA1* would increase mobility rates compared to cells with only vector added.

Conclusion

- As expected, replacement of *BRP1* with *URA3* decreased Ty1 mobility rates, showing that lower expression of the Pma1 proton pump impacts retrotransposition. More trials will be carried out to better determine the magnitude of this effect.
- The unexpected results found in the plasmid experiment could be due to the plasmid being low-copy and not having enough overexpression - use of a plasmid with a higher copy number may provide a better test.
- The unexpectedly high mobility of Ty1 in the vector strain could be the cause of not seeing the expected result. Conducting more trials will address this issue.