Regulation of Human *HRAS1* Minisatellite Stability During Stationary Phase

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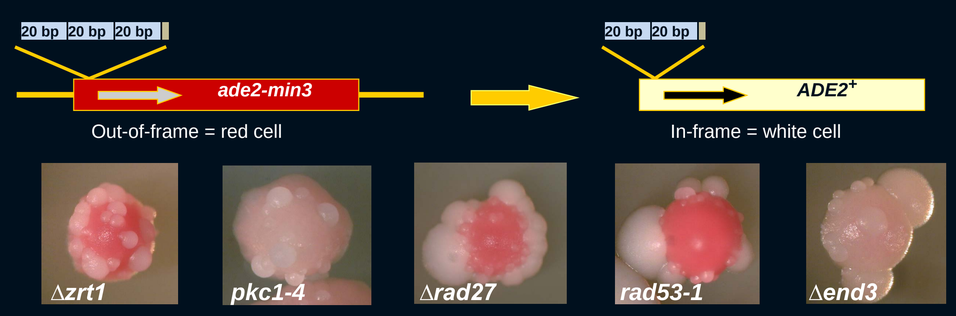
Abstract

Minisatellites are repetitive tracts of DNA with repeat units ranging from 16-100 base pairs in length. They are stable during mitosis but display changes in repeat number and order after meiosis. Rare alleles of minisatellite tracts thought to arise from repeat instability are associated with human diseases, including cancer, diabetes, and epilepsy. The stability of minisatellites in non-proliferating stationary phase cells is not well understood. Previous work has shown that the zinc transporter *ZRT1*, the checkpoint gene *RAD53*, the DNA repair gene *RAD27*, the endocytosis gene *END3*, and the protein kinase *PKC1* regulate the stability of minisatellites in *S. cerevisiae* during stationary phase. We inserted the human minisatellite associated with *HRAS1* into the *ADE2* gene to determine how its stability is regulated during stationary phase. Loss of *ZRT1*, *RAD27*, or *RAD53* destabilized the minisatellite; loss of *PKC1* or *END3* had no effect. This work contributes significantly to our understanding of repeat stability and genome stability during stationary phase; this has important implications for human genome stability, since most human somatic cells are non-proliferating.

Mutations Effecting Minisatellite Stability

1



The *ade2-min3* cassette consists of three identical 20 base pair repeat units inserted into an *Xba*I site in the *ADE2* gene of *S. cerevisiae*. This insertion disrupts the *Xba*I 4-base overhang and knocks the *ADE2* gene out of the correct reading frame, forming red colonies. Changes in the number of repeat units knocks the *ADE2* gene back into the correct reading frame, producing a white sector or papillation on the colony. Sectors indicate mitotic instability, while papillations called "blebs" indicate instability during stationary phase. Five genes were shown to have an effect on minisatellite stability in the *ade2-min3* cassette. These genes are the zinc transporter gene *ZRT1*, the protein kinase *PKC1*, the checkpoint gene *RAD53*, the DNA repair gene *RAD27*, and the endocytosis gene *END3* (Kelly *et al.* 2007). The color segregation phenotype caused by minisatellite instability during stationary phase is called blebbing.. After minisatellites alterations occur, white papillations or blebs form on the red colony. Above are pictures of blebbing in each of the *ade2-min3* strains.

Photos courtesy of M. Kelly

HRAS1 Minisatellite

2

Repeat Type	Sequence
1	ggcgtcccctggaGagaagggCgagtgt
2	ggcgtcccctggaGagaagggGgagtgt
3	ggcgtcccctggaCagaagggGgagtgt
4	ggcgtcccctggaCagaagggCgagtgt

(+14 and +22 mark the positions of the variable bases)

HRAS1 Minisatellite and Cancer

- Breast
- Colon
- Ovarian
- Brain
- Urinary Tract
- Acute Leukemia

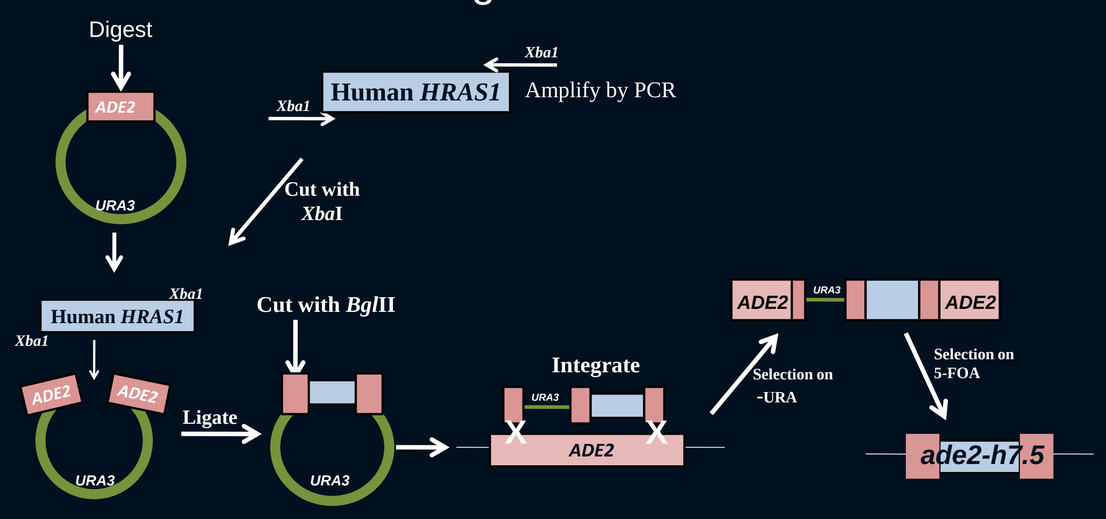
***HRAS1* - *7.5* repeat minisatellite**

In the human *HRAS1* minisatellite there are 4 main repeat types, with each repeat unit being 28 base pairs in length. These repeats are different from one another at two positions, the +14 and +22, containing either a G or C. Type 1 repeats consist of a G at +14 and a C at +22, while Type 2 repeats have 2 Gs. Type 3 repeats have a C at +14 and a G at +22, and Type 4 repeats have two Cs. Rare alleles of the *HRAS1* minisatellite are associated with various cancers, listed above (Krontiris *et al.* 1993).

Figures courtesy of M. Kelly

Constructing the *ade2-h7.5* strain

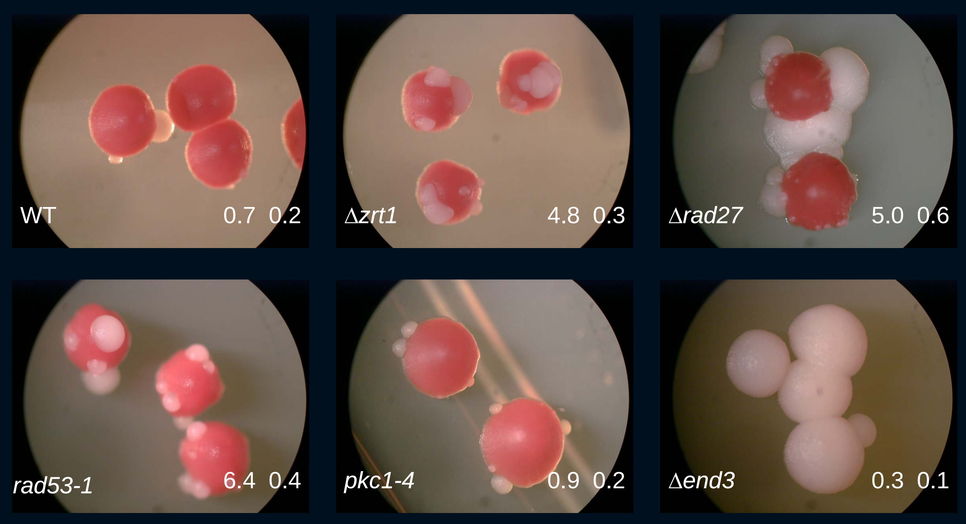
3



The original plasmid pEAS8 contained the ADE2 gene. We digested this plasmid with the endonuclease *Xba*I. Oligos complimentary to a unique sequence surrounding the human *HRAS1* minisatellite repeats with *Xba*I flanking ends were used to amplify the *HRAS1* minisatellite by PCR. We digested the human *HRAS1* with *Xba*I. The amplified region was then ligated into the *ADE2* region of the plasmid pEAS8. The plasmid now containing the *ADE2* and *HRAS1* regions was cut using the endonuclease *Bgl*II. The linearized plasmid was transformed into the *ADE2* gene of *S. cerevisiae* and was selected on 5-fluoroorotic acid to remove the plasmid backbone and produce the *ade2*-h7.5 strain. PCR and sequencing comparing the *ade2*-h7.5 to the *ADE2* parental strain verified the insertion and transformation.

ade2-h7.5 Strains Blebbing Quantitations

4

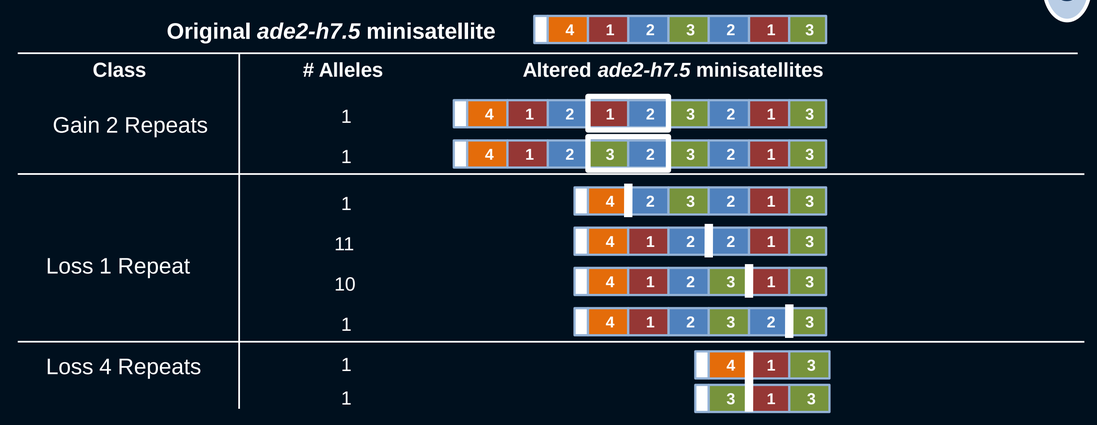


Above are pictures and quantitations of blebbing for each of the *ade2*-h7.5 strains. All colonies were grown 3 days at 30 C then transferred to room temperature for an additional 4 days. Mean number of blebs per colony 95% confidence interval were calculated for each strain by surveying at least 100 colonies over 3 independent trials. The *ZRT1*, *RAD27*, and *RAD53* mutants displayed a significantly higher number of blebs per colony than the wild type (as determined by non-overlap of the 95% confidence intervals.) However, the *PKC1* and *END3* mutants did not display a significantly elevated level of blebbing.

Photos courtesy of M. Kelly

ade2-h7.5 Δ*zrt1* Minisatellite Alterations

5



The figure above illustrates the minisatellite alterations that occurred in the *ade2-h7.5* Δ*zrt1* strain. Sequencing of the *ade2-h7.5* minisatellite tract from 27 white bleb isolates of the Δ*zrt1* strain verified the exact alterations that the *ade2-h7.5* Δ*zrt1* strain undergoes. There are 3 classes of alterations in the *ade2-h7.5* Δ*zrt1* strain: gain of 2 repeats, loss of 1 repeat, and loss of 4 repeats. The number of alleles and the sequence of repeat types for each class is shown.

Figures courtesy of M. Kelly

ade2-h7.5 Δ*zrt1* Recombination Mutants

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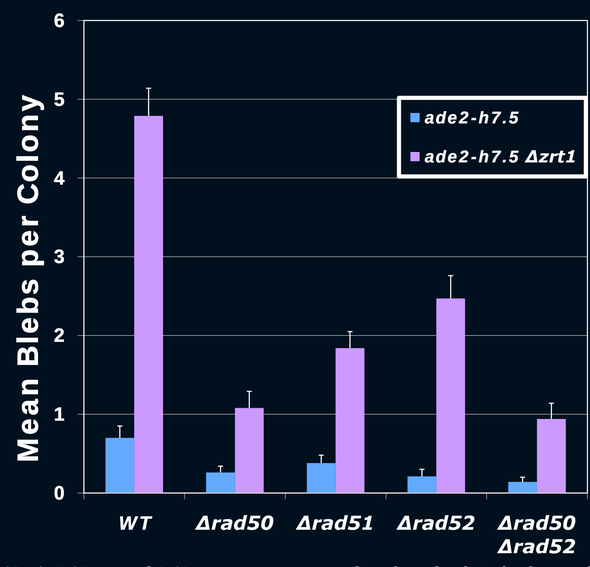

Recombination mutants of *RAD50*, *RAD51*, and *RAD52* were constructed in the *ade2-h7.5* background. Blebbing was quantitated using the same method as described above. Error bars represent the 95% confidence interval for the mean. The *ade2-h7.5* Δ*zrt1* strain showed significantly higher blebbing than the *ade2-h7.5* in each of the recombination mutants. Blebbing in both the *ade2-h7.5* Δ*zrt1* and *ade2-h7.5* strains was significantly reduced when recombination factors were knocked out. This indicates that *ade2-h7.5* minisatellite alterations are dependent on the recombination factors *RAD50*, *RAD51*, and *RAD52* .

Figure courtesy of M. Kelly

Conclusions

- The human *HRAS1* minisatellite can be destabilized by loss of *ZRT1*, *RAD27*, and *RAD53*
- Alterations in minisatellites include both gains and losses of repeats
- Minisatellite alterations occur by homologous recombination

References

Kelly, Jauert, Jensen, Chan, Truong, and Kirkpatrick (2007) 'Zinc Regulates the Stability of Repetitive Minisatellite DNA Tracts During Stationary Phase', Genetics 177: 2469-2479.

Krontiris S, T. G., B. Devlin, D. D. Karp, N. J. Robert and N. Risch, 1993 An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N Engl J Med 329: 517-523.