Rtt105 Regulates Cellular Location of a Retrotransposon Gag Protein in Yeast

Haley Smith and Jill B. Keeney

Dept of Biology Juniata College Huntingdon, PA 16652

Abstract

The Rtt105 protein (Regulator of Ty1 transposition 105) of *Saccharomyces cerevisiae* has been shown to function as a chaperone for Replication Protein A (RPA), which protects ssDNA regions at replication forks during DNA replication. Rtt105 is thought to escort RPA to the nucleus and facilitate loading at replication forks. RTT105 was first identified in a screen for host regulators of Ty1 transposition and thus named Regulator of Ty Transposition. Ty1 is a retroelement that is transcribed to RNA and then reversed transcribed to DNA and integrated into the host genome. Our lab has found that in wild type yeast cells, the Ty1 Gag protein is located in cytoplasmic foci; however, when cells are stressed with hydroxyurea (HU), the Ty1 Gag is transported to the vacuole. In the absence of Rtt105 protein, Ty1 Gag protein remains in cytoplasmic foci under stress, suggesting that Rtt105 may also be a chaperone for Ty1 Gag. The goal of this project has been to mutate Rtt105 in multiple positions and to determine if the transport of the Tyl Gag protein to the vacuole is inhibited by these mutations. Microscopy was used to localize fluorescently tagged Gag protein within the yeast cell. With many of these mutant Rtt105 proteins, Ty1 Gag stayed in the cytoplasmic foci in both stressed and non-stressed conditions. This data supports the hypothesis that Rtt105 plays a role in transporting the Ty1 Gag protein within the cell.

.

Background

- Rtt105 cellular function not entirely known; deletion of Rtt105 decreases Ty1 transposition
- Tys (transposons in yeast) aide in understanding how retrotransposons copy and integrate themselves into the host genome. Ty1 is a model system to study the role of host genes in retroviral function.
- Ty1 Gag protein forms the viral particle.
- Under stress (addition of hydroxyurea) Ty1 Gag relocates from cytoplasmic foci to the vacuole.
- Deletion of Rtt105p prevents relocation to the vacuole.
- Studying localization of the Gag protein may increase understanding of viral/host interactions.

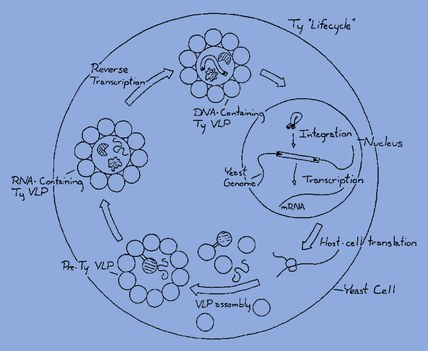

Fig 1. Ty1 life cycle. RNA from the transcribed Ty1 element is packaged into virus-like particles (VLPs) formed of Gag protein. Within VLPs, the RNA is reverse transcribed to cDNA and is subsequently integrated into the host genome

Results

- Gag protein followed by transformation of rtt105-deleted yeast cells with a plasmid carrying Ty1 Gag-RFP fusion protein.
- Test strains carried a second plasmid with wild type *RTT105* (Fig 2 A/B), an empty vector (Fig 2 C/D) or mutant *rtt105 (*Fig2 E-N*)*
- Rtt105p moves Ty1 Gag to vacuole under HU stress (Fig 2 B)
- In absence of Rtt105p, Ty1 Gag stays as foci upon HU stress (Fig 2 D)
- A panel of *rtt105* mutants was tested for Ty1 Gag transport (Fig2 E- N)

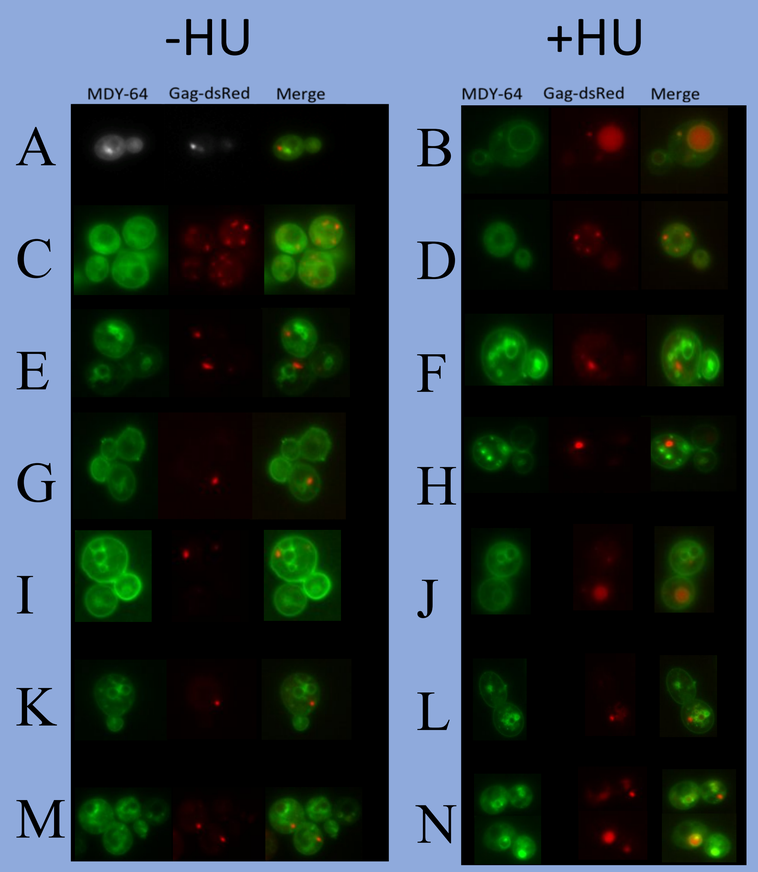

Figure 2. (A-N) Fluorescent microscopy (100x) using MDY-64 stain vacuoles (green channel) and Ty1 Gag-RFP (red channel). In each set, MDY-64 (green) is left panel, Gag-RFP (red) in center panel, and merged is the right panel. The two channels were merged to visualize the localization of the Ty1 Gag protein. Samples were grown in glucose to log phase, divided, and grown without (left hand column) or with (right hand column) hydroxyurea for 4 hours. (A) Wild type Rtt105 strain, -HU; Ty Gag as foci. (B) Wild type Rtt105 strain, +HU; Ty Gag in vacuole. (C) Rtt105 deletion, -HU; Ty Gag as foci. (D) Rtt105 deletion, + HU. Ty Gag as foci. (E) Rtt105 E185V, -HU; Ty Gag as foci. (F) Rtt105 E185E, +HU; Ty Gag as foci. (G) Rtt105 M155T, -HU; Ty Gag as foci. (H) Rtt105 M155T, +HU; Ty Gag as foci. (I) Rtt105 K178N, -HU; Ty Gag as foci. (J) Rtt105 K178N, +HU; Ty Gag in vacuole. (K) Rtt105 E180G, -HU; Ty Gag as foci. (L) Rtt105 E180G, +HU; Ty Gag as foci. (M) Rtt105 M107*, -HU; Ty Gag as foci. (N) Rtt105 M107*, +HU; Ty Gag as foci and Ty Gag in vacuole.

Table 1. Summary of Rtt105p mutant phenotype

Mutant location (Rtt105p is 208 AA)	AA change	Ty1 Gag location +HU	Gal Transposition phenotype*
Wild type (Fig 2A/B)	NA	Vacuole	5
Deletion (Fig 2 C/D)	NA	Foci	1
S34R	Non-conservative	Vacuole	TBD
T42A	Non-conservative	Foci and Vacuole	TBD
E49D	conservative	Vacuole	TBD
E50G	Non-conservative	Foci	TBD
K52R	Conservative	Foci	2
N57I	Non-conservative	Foci	TBD
M107* (Fig 2 M/N)	NA	Foci and Vacuole	TBD
T110N	Non-conservative	Foci	TBD
R112H	Conservative	Foci	TBD
Q132P	Non-conservative	Foci	2
M155T (Fig 2 G/H)	Non-conservative	Foci	TBD
L167S	Non-conservative	Foci	TBD
E177D	conservative	Vacuole	TBD
K178N (Fig 2 I/J)	Non-conservative	Vacuole	TBD
E180G (Fig 2 K/L)	Non-conservative	Foci	TBD
K183N	Non-conservative	Vacuole	TBD
E185V (Fig 2 E/F)	Non-conservative	Foci	TBD
N205Y	Non-conservative	Foci	TBD

* scale of 1-5: 1 very low; 5 wild type

Discussion/Future Plans

- In most mutations of Rtt105 (stressed and non-stressed conditions) the Ty1 Gag protein stayed in the cytoplasmic foci unlike the wild type.
- Future studies will be looking at more specific mutations within Rtt105 and look at the effects of these mutant R1105 molecules on transposition.
- A recently published study showed that Rtt105p may be involved in transport of nuclear proteins, suggesting that Rtt105p is a transport-related protein (EMBO J. 2018 Sep 3;37(17))

Acknowledgements

Devin Conaway and Mark Weir rtt105 mutant construction
Marina Segura strain construction.