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Electronic Supplementary Material

Identification of Yeast Factors Involved in the Replication of Mungbean Yellow Mosaic India Virus Using Yeast Temperature-Sensitive Mutants

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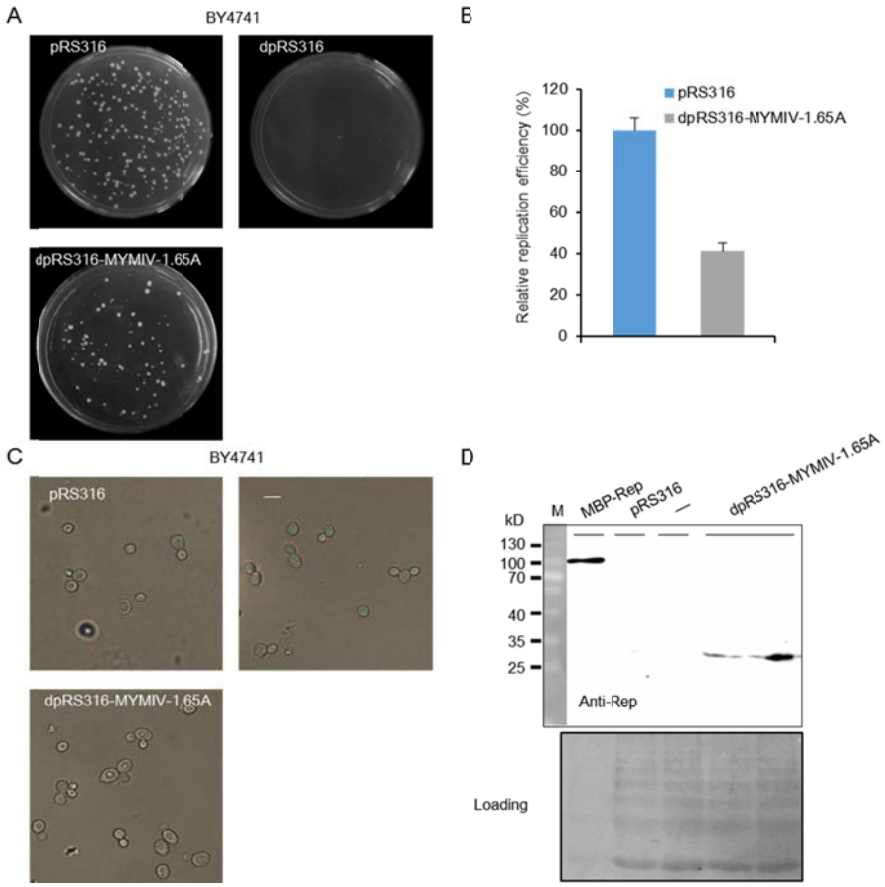

Fig. S1 The replication of Mungbean yellow mosaic India virus (MYMIV) in yeast cells. (**A**) Representative plates comparing colonies of the transformed reference BY4741 yeast strain (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) with plasmid pRS316, dpRS316, or dpRS316-MYMIV-1.65A. Photographs were taken at 5 days post transformation. (**B**) Relative replication efficiency of the various constructs relative to pRS316, which was given a value of 100%. (**C**) Cell morphology of various colonies transformed with pRS316, dpRS316-MYMIV-1.65A, respectively. No transformed BY4741 as a control (-). (**D**) Western blot assay of the expression of replication initiator protein (Rep) in yeast cells, the anti- MYMIV Rep antiserum was used to detect the expression of MYMIV Rep. Coomassie Brilliant Blue (CBB)-stained the total protein extracted from yeast cells was used as a loading control.

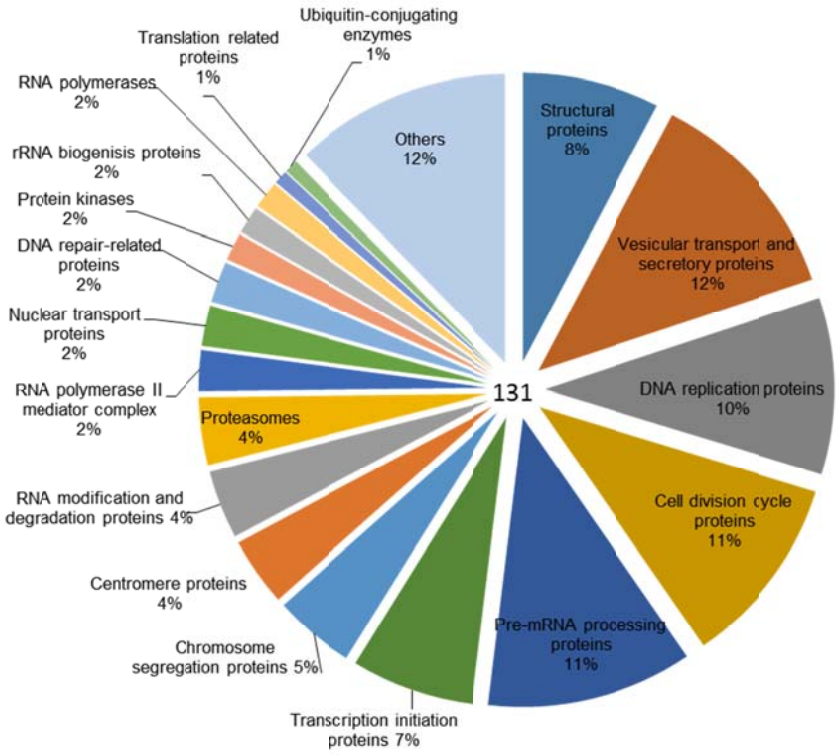

Fig. S2 Classification of essential yeast genes affecting MYMIV replication. The ts mutant library screens resulted in the identification of 131 unique yeast genes that specifically affect MYMIV replication. The identified host genes encoding proteins were grouped into 20 categories based on their known cellular/biochemical functions. The percent values mean the number of each grouped genes encoding proteins compared to the screend 131 unique yeast genes encoding proteins.

Material and Methods

Generation of Plasmid Constructs

Wild plasmid pRS316, and recombinant plasmids dpRS316, dpRS316-MYMIV-1.65A were described previously (Li *et al*. 2015). Briefly, the ARS (autonomous replicating sequence) was removed from the shuttle plasmid pRS316 by PCR amplification using the primer pair (dARSH4/NheI/F and dARSH4/*Nhe*I/R), then digested with *Nhe* I, and self-ligated to form the plasmid dpRS316. The recombinant plasmid containing the viral genome of MYMIV, a 1795 bp fragment (0.65A) of MYMIV DNA-A was obtained by digesting the infectious clone plasmid pCambia2300-MYMIV-1.65A with *Hin*d III and *Bam*H I. Then the 0.65A fragment was inserted into dpRS316 to obtain the recombinant plasmid dpRS316-MYMIV-0.65A. A full copy of MYMIV DNA-A was generated by digesting pCambia2300-MYMIV-1.65A with *Bam*H I, and then inserted into dpRS316-MYMIV-0.65A to ultimately obtain the recombinant yeast shuttle plasmid dpRS316-MYMIV-1.65A.

Yeast Temperature-sensitive Mutants

The temperature-sensitive (ts) mutant library of essential genes in *S. cerevisiae* has been constructed in Boone's lab (Li *et al*. 2011). This ts mutant library currently contains 787 yeast haploid mutants and represents 45% of essential yeast genes (497 out of 1101 essential yeast genes), of which 30% are represented by multiple ts mutants. All of the alleles are integrated into their native genomic locus in the BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) reference strain. The alleles are also linked to a kanMX selectable marker (Li *et al*. 2011).

Yeast Transformation and Cultures

The yeast ts mutants were transformed by lithium acetate/PEG mediated method of transformation as described previously (Nawaz-ul-Rehman *et al*. 2013). The recombinant yeast shuttle plasmids pRS316, dpRS316 and dpRS316-MYMIV-1.65A were transformed into yeast ts mutants and BY4741 reference strain, respectively.

The transformants were plated on synthetic complete media lacking uracil (SC/-Uracil) and cultured at permissive temperature and semi-permissive temperature (usually 4–5 ℃ below the nonpermissive temperature), respectively, according to the ts mutants' nonpermissive temperature. Photographs were taken at 5 days post transformation.

Western Blot Analysis

Yeast cells transformed with pRS316, dpRS316-MYMIV-1.65A, respectively, and no-transformed BY4741 cells were collected by centrifugation. The crude protein was extracted from the collected yeast cells as described previously (Panavas *et al*. 2004). Immunoblotting was performed with anti-MYMIV Rep

antiserum, followed by goat anti-rabbit secondary antibody conjugated to horseradish peroxidase. Blotted membranes were washed 5 times and visualized by chemiluminescence.

References

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