Involvement of the INO80 and SWR1 Complexes in Chromosome Segregation

Jesús Moreno, Jacob Ginter, Md. Riajul Hossain and Inés Pinto

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Introduction

Faithful chromosome segregation is an essential property of cell division, required for the maintenance of the genome's integrity. The main goal of this work is to gain an understanding of the role that chromatin and chromatin remodeling complexes have during mitotic chromosome segregation. In an effort to evaluate the proteins that are involved in ploidy maintenance, we carried out a genetic screen of the non-essential deletion library for genes that when mutated caused ploidy increase. Among the mutants that increased ploidy, we encountered members of the INO80 and SWR1 complex. Both ATP-dependent chromatin-remodeling complexes participate in a variety of biological processes including transcription, DNA repair, DNA replication, and maintenance of chromosome integrity. INO80 catalyzes the eviction of the H2A.Z histone variant replacing it with H2A. This complex is comprised of 15 subunits, and their specific contribution to chromosome segregations remains largely unknown. The INO80 complex has been implicated in the maintenance of ploidy through the characterization of mutations of the genes encoding the Ies6 and Ino80 subunits [1], which result in a clear ploidy increase. The SWR1 complex catalyzes the exchange of H2A for H2A.Z. The yeast SWR1 complex is comprised of 14 subunits. The Swr1 subunit creates the scaffold of the complex, and is essential for its enzymatic activity.

To evaluate the contribution of each subunit to chromosome segregation, we tested deletion mutants of all the non-essential subunits of both complexes, as well as a ts allele of *ARP4* for benomyl sensitivity and increase-in-ploidy phenotypes. We also analyzed genetic interactions between the ploidy-increase causing alleles and *SGO1,* the gene encoding shugoshin, required for sensing tension and mitotic chromosome stability. Finally, we assessed by ChIP the association and activity of Ino80 with pericentromeric regions in the presence and absence of several INO80 subunits that when mutated cause an increase in ploidy.

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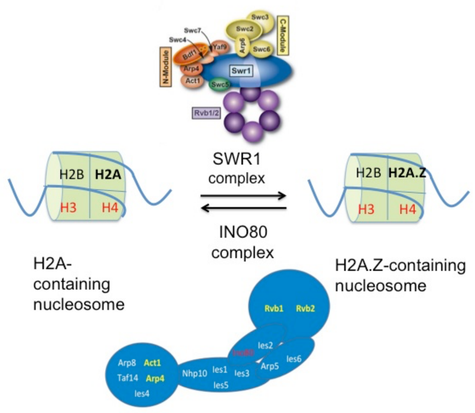
Background



The INO80 complex

- Histone exchange activity: H2A.Z-H2B for H2A-H2B dimer

The SWR1 complex

- Histone exchange activity: H2A-H2B dimer for bulkier H2A.Z-H2B
- Deletion of Swr1 subunit effectively removes the scaffolding for the SWR complex.

- Both complexes involved in DNA repair, DNA replication, transcription, and chromosome stability.

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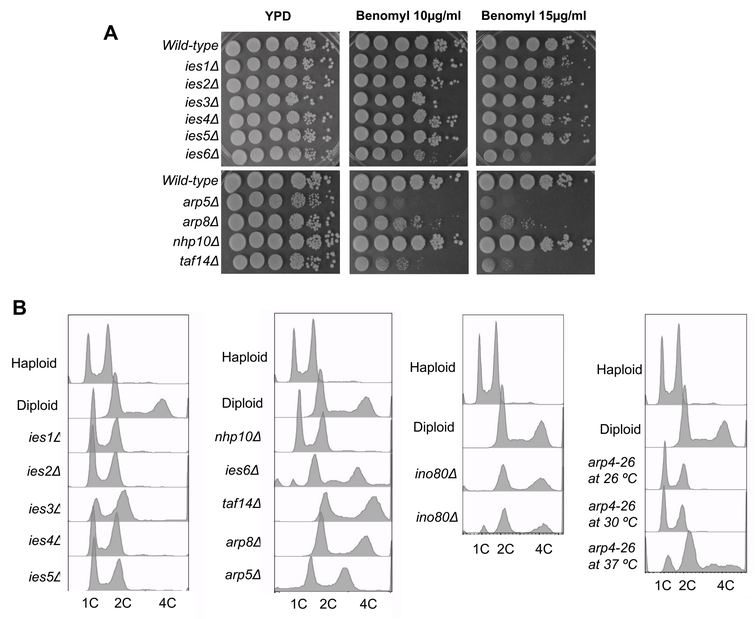
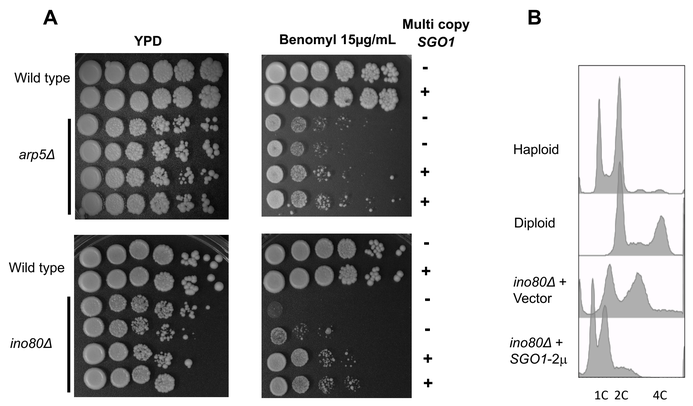


Figure 1. Mutations in some subunits of the INO80 complex display benomyl sensitivity and ploidy increase. (A) YPD agar plates with or without benomyl were used to plate ten-fold dilutions of the indicated strains, starting with 10^8 cells/ml (first column). **(B)** DNA content of the indicated strains with determined by flow cytometry of exponentially growing cells.



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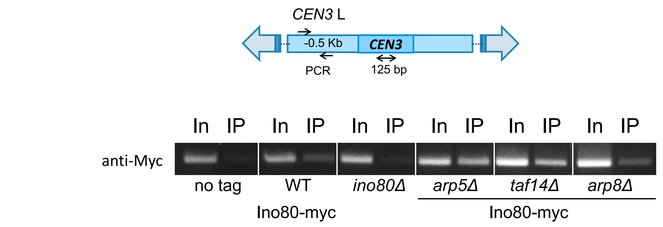

Figure 3. Chromatin immunoprecipitation (ChIP) of Ino80-Myc tagged strains. Preliminary data shows association of Ino80 with pericentromeric regions (CEN3 L) even in the absence of the INO80 subunits Arp5, Arp8 and Taf14. ChIP was carried out with anti-Myc antibody or anti-H2AZ antibody. Primers used for PCR are shown on the top diagram.

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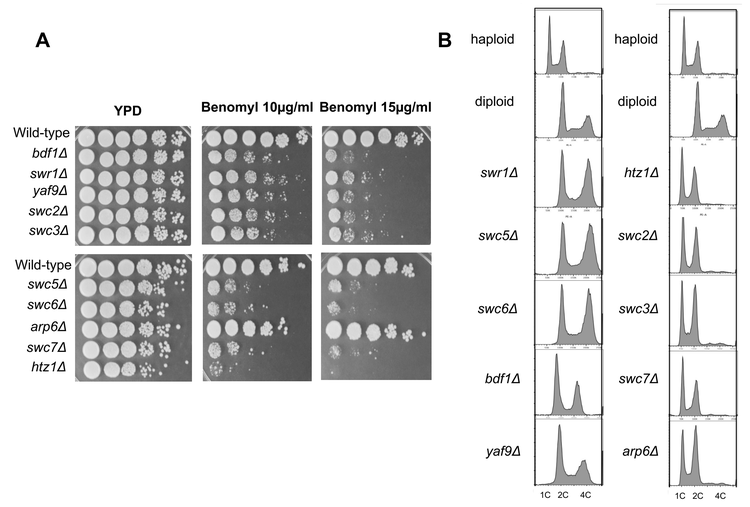
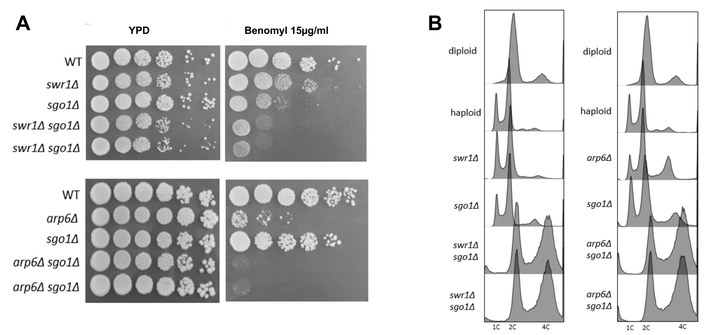


Figure 4. Mutations in some subunits of the SWR1 complex display benomyl sensitivity (A) and ploidy increase (B). Methodology as in Fig.1.



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Conclusions

- Specific subunits of the INO80 complex are involved in the maintenance of ploidy and chromosome stability (Ino80, Ies6, Taf14, Arp5, Arp8, Arp4^{ts}).
- Neither Arp5, Arp8, nor Taf14 are required for recruitment of Ino80 to pericentromeric regions. We are currently determining whether the levels of pericentromeric H2A.Z are affected in these various strains.
- Overexpression of *SGO1* alleviates the missegregation impairment of *INO80* mutants, likely by re-establishing bi-orientation.
- Similarly to the results seen with mutations in the INO80 complex, deletion of only five subunits of the SWR1 complex caused diploidization (*swr1Δ, swc5Δ, swc6Δ, bdf1Δ,* and *yaf9Δ*).
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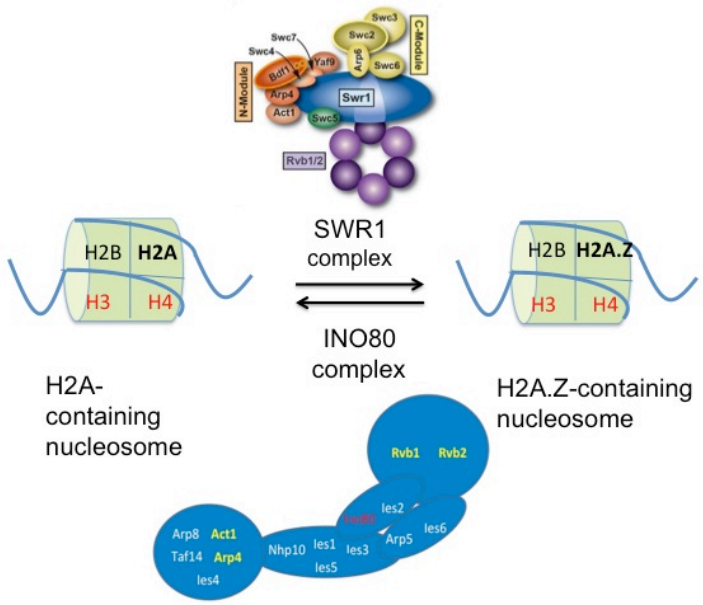

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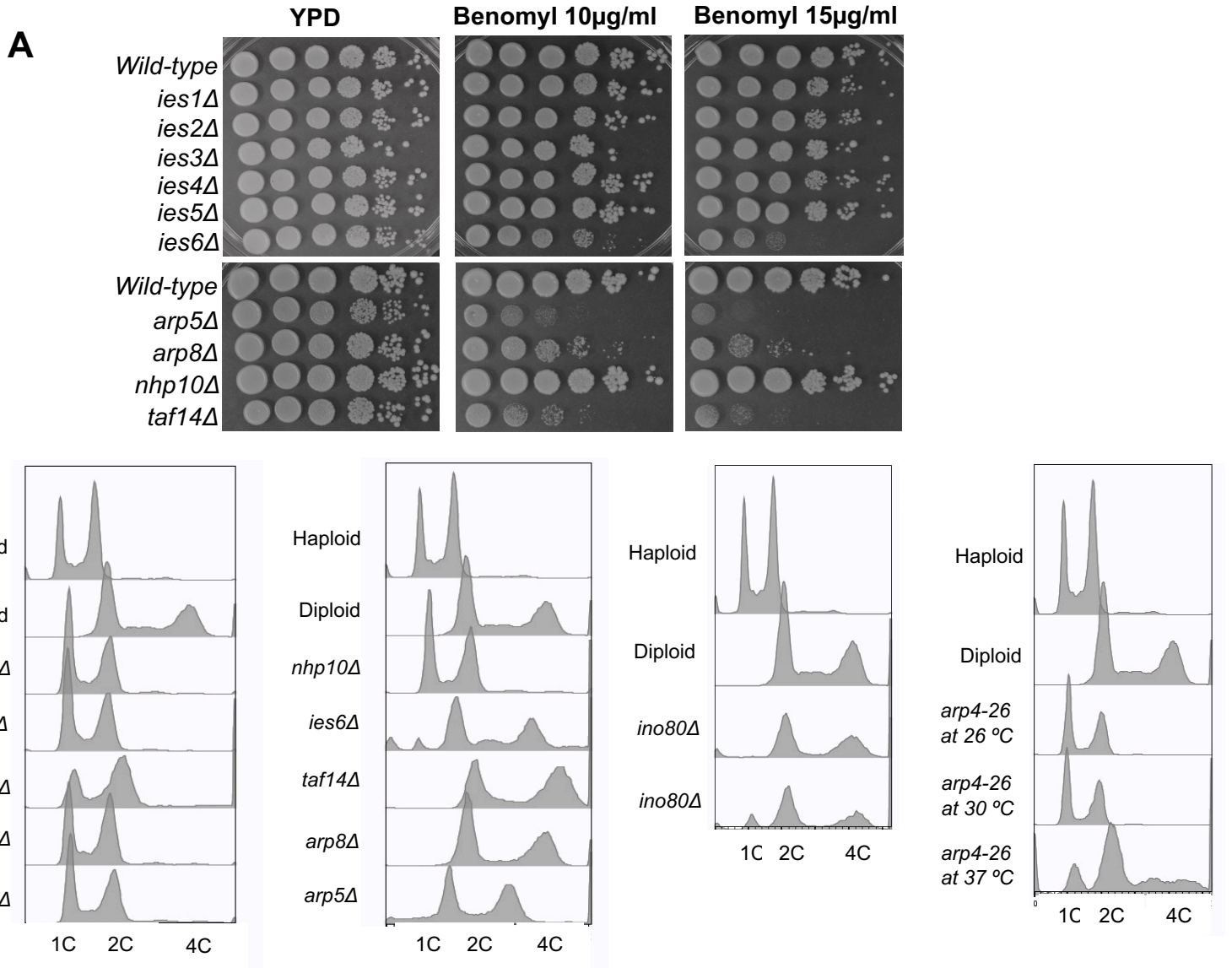

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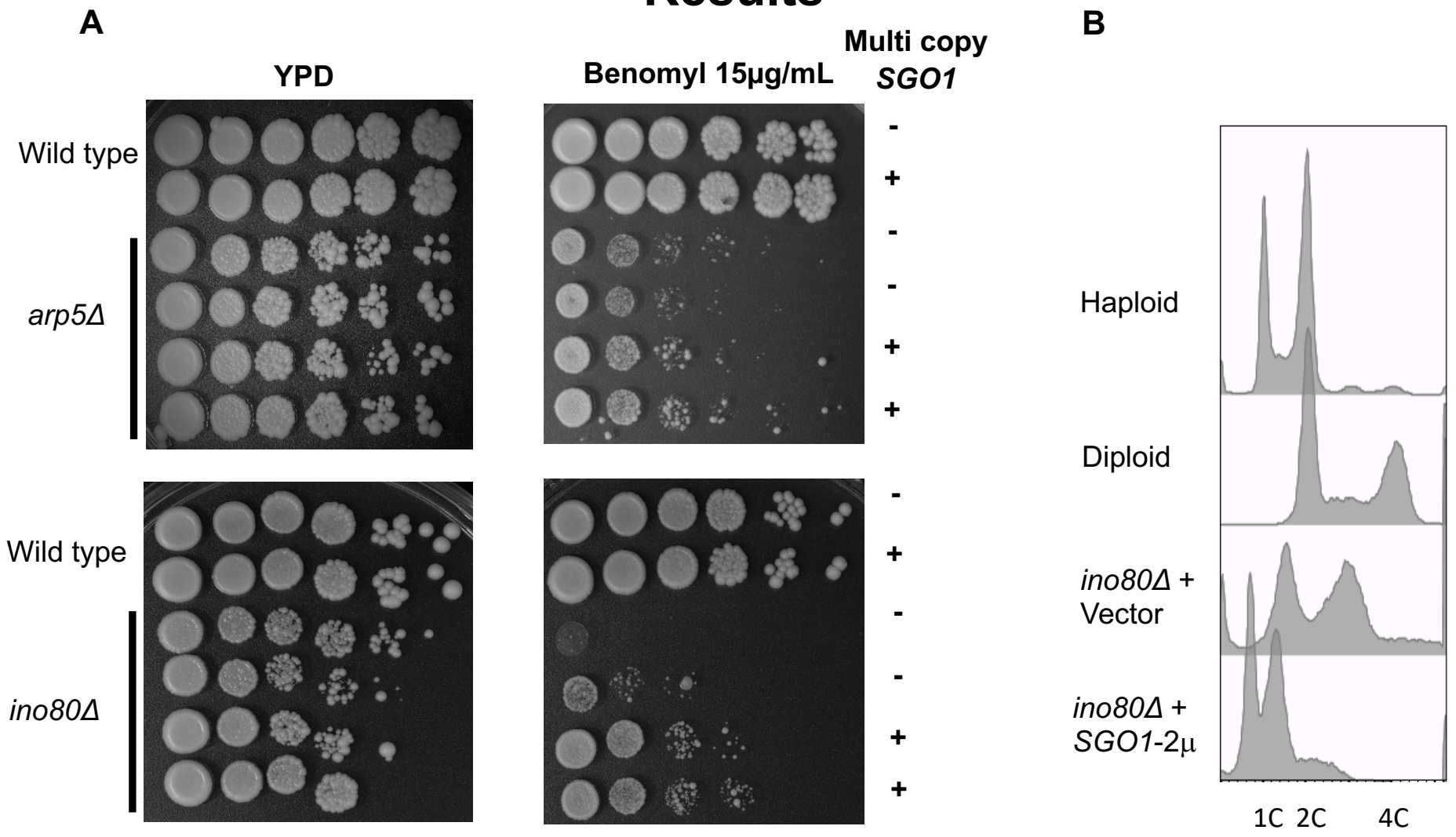

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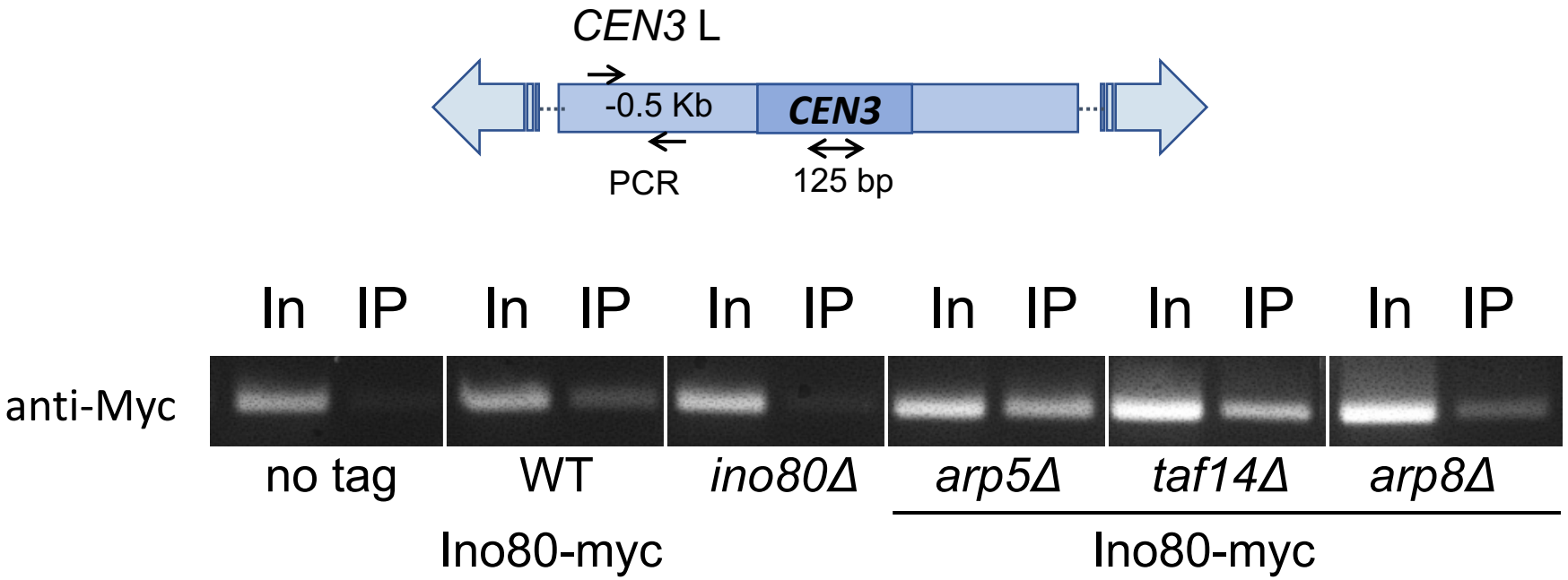

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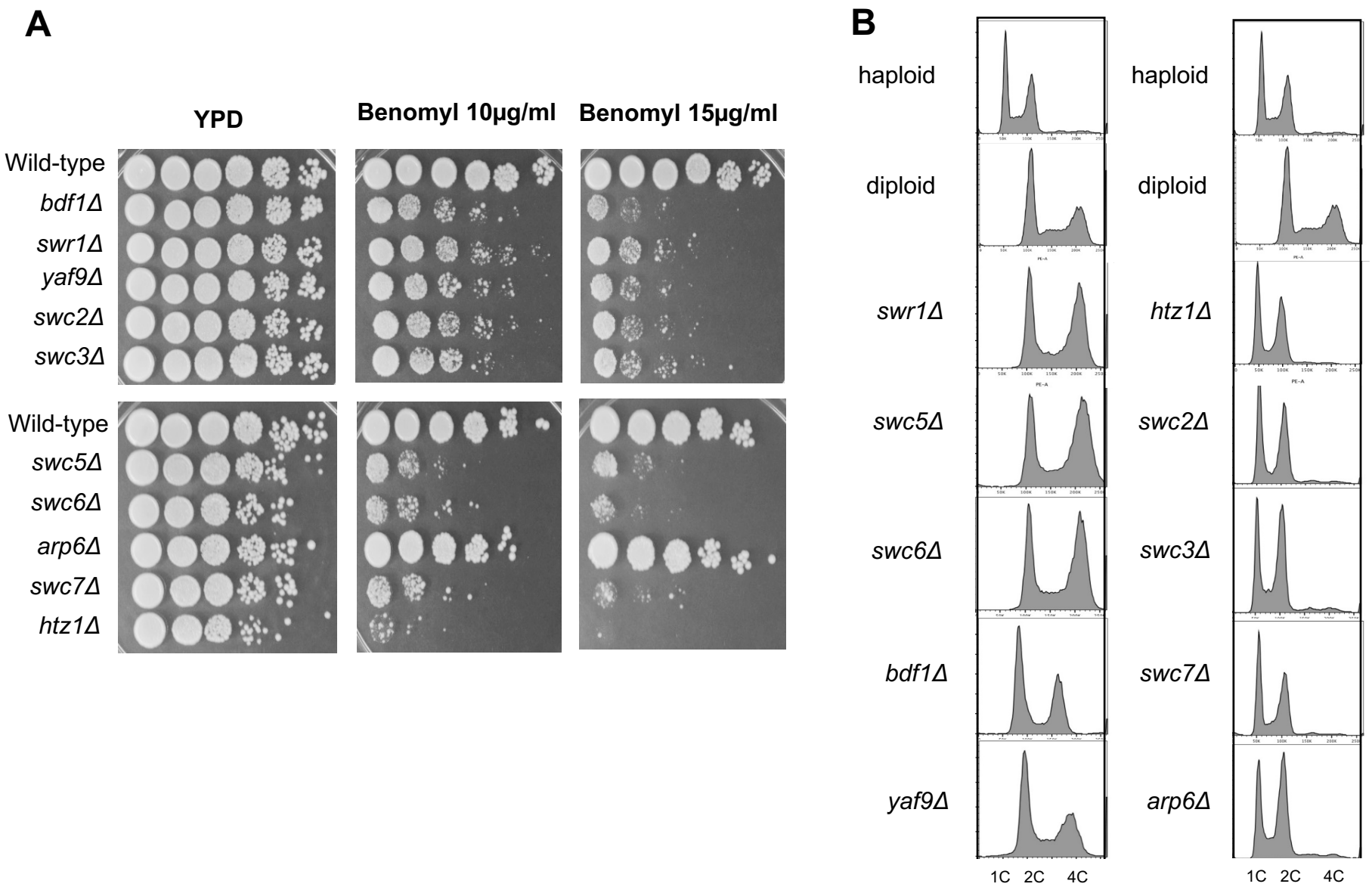

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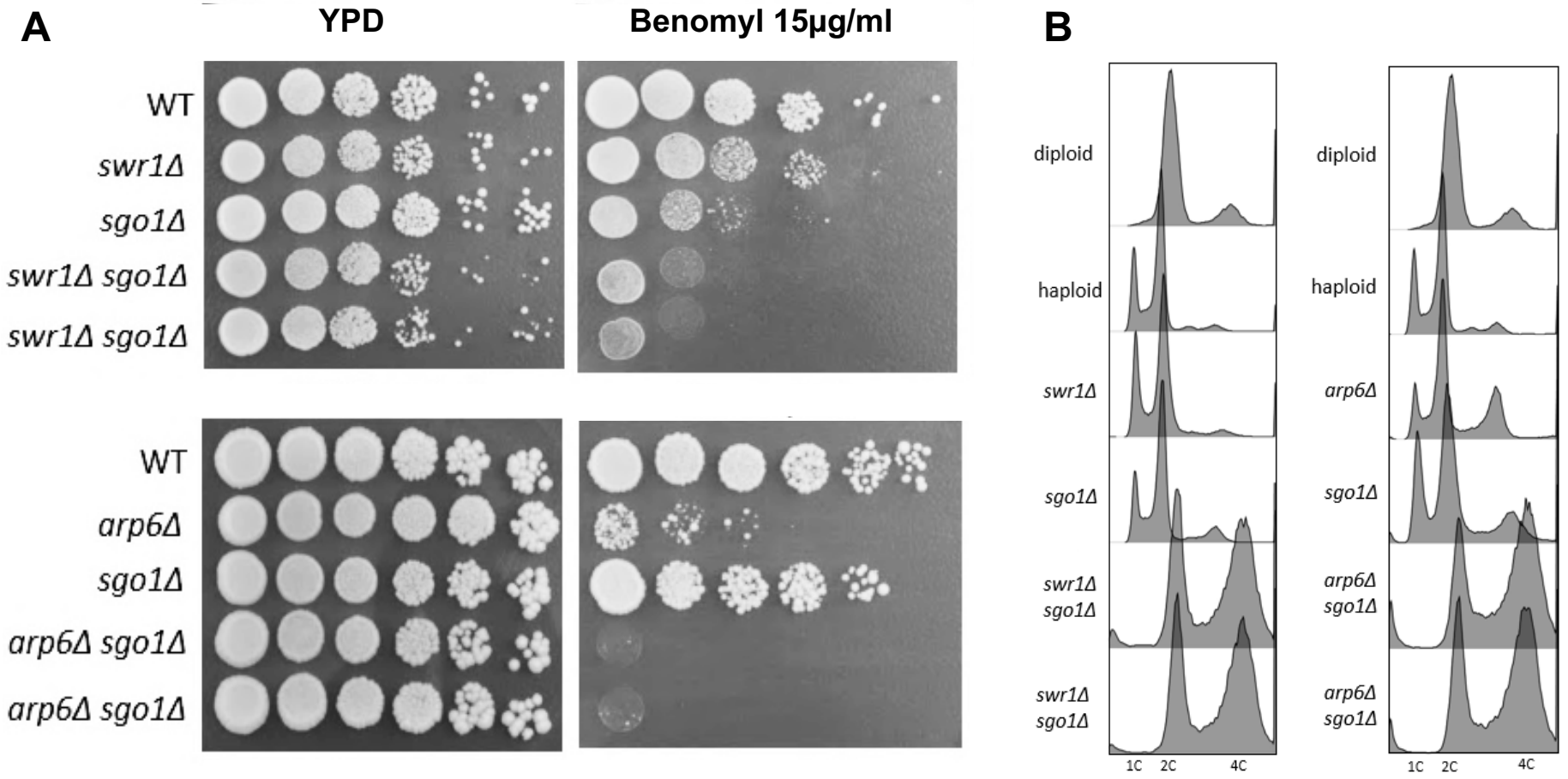

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