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Drug-Induced Haploinsufficiency of Fission Yeast Provides a Powerful Tool for Identification of Drug Targets

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Abstract Genome-wide systematic deletion mutants were generated using a PCR-based targeted mutagenesis of *Schizosaccharomyces pombe*. In a drug-sensitivity assay using thiabendazole (TBZ), an inhibitor of microtubule assembly, a heterozygous *nda2* mutant ($nda2^+/nda2^-$), deleting one copy of *nda2* encoding the microtubule subunit alpha1 demonstrated a distinct sensitivity to TBZ, indicating TBZ-induced haploinsufficiency. This result suggests that profiling drug-induced haploinsufficiency can be exploited to identify target genes for drugs and discover new drugs.

Key words: *Schizosaccharomyces pombe*, PCR-based targeted mutagenesis, haploinsufficiency, drug target

Schizosaccharomyces pombe has served as an excellent model organism for the study of cell cycle control, mitosis and meiosis, DNA repair and recombination, checkpoint controls, and genome stability. The completion of the full genome sequence has opened the era of the functional genomics of *S. pombe*. The 13.8-Mb genome of *S. pombe* contains the smallest number of 4,824 protein-coding genes recorded for eukaryotes [12]. Since disruption of the gene in *S. pombe* causes phenotypic changes that are easily detectable and analyzable, the collection of individual deletion mutants can be a valuable resource for investigating the function of genes in yeasts and humans. Targeted mutagenesis by a PCR-amplified deletion cassette has already been developed in yeast [2, 7, 9], while the genome-wide deletion mutants of *Saccharomyces cerevisiae* have been constructed and analyzed in parallel for gene function [10]. As such, the current report explains the construction of genome-wide systematic deletion mutants generated by a PCR-based targeted mutagenesis as a tool for functional genomics and drug discovery.

Stable nonsporulating fission yeast diploid SP286 (h^+/h^+, *ade 6-M210/ade 6-M216 ura 4-D18/ura 4-D18 leu 1-32/leu 1-32*) was used as the parental strain for the construction of the deletion mutants. The deletion cassettes contained a kanMX4 module (GenBank #AJ002680) to give resistance to G418 for selection, two tag sequences to label the deletion mutants, two universal primers to amplify the tag sequences, and 80 bp of ORF for homologous recombination at the target site. A pool of forty-thousand 20-base oligonucleotides was designed as tag sequences using a computer algorithm based on several parameters, and two tags were allotted to each gene deletion cassette as an uptag and a downtag. Universal primers of 20-mer oligonucleotides were used to amplify the tag sequences in a parallel analysis of mutants using a high-density oligonucleotide DNA chip.

The deletion cassettes were amplified by a four-round PCR (Fig. 1) and used to transform the parental diploid SP286 using the lithium acetate method [8, 11]. The transformants grown in YE plates containing G418 were

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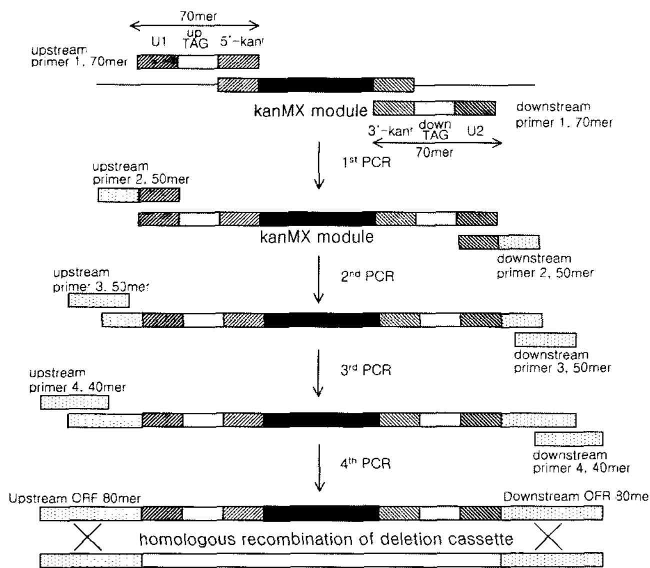

Fig. 1. Amplification of the deletion cassette.
The deletion cassettes were amplified by a four-round PCR using the plasmid pFa6-kanMX4 as a template. Deletion cassettes containing the kan^r gene, tag sequences, and universal primers were amplified in the first-round PCR. The length of the ORF for homologous recombination was extended to 80 bp in the second, third, and fourth PCR, and the previous PCR product was used as the template DNA. All oligonucleotides were synthesized and generously provided by Bioneer Corp.

examined for the deletion of the target gene by colony PCR using common primers in a kanMX module. Consequently, a total of 1,925 heterozygous deletion mutants were constructed, with a deletion frequency of 2-100% and average deletion efficiency of 16%.

Haploinsufficient loci define the set of genes whose dosages and functions are critical to an organism. Some genes have been identified as haploinsufficient in humans, *D. melanogaster*, *C. elegans*, and *S. cerevisiae*, because they result in developmental abnormalities or other severe diseases [3, 6]. Therefore, drug-induced haploinsufficient phenotypes of *S. pombe* were examined using thiabendazole (TBZ), an inhibitor of microtubule formation that affects the progression of mitosis in fission yeast. Fission yeast has two alpha-tubulin genes and one beta-tubulin gene. Gene disruption experiments have shown that the alpha 1-tubulin gene (*nda2*) is essential, whereas the alpha 2 gene is dispensable [1]. In the current study, the alpha 2-disrupted cells exhibited an increased sensitivity to the antimicrotubule drug thiabendazole, while the alpha 1 [cold-sensitive (cs)] and alpha 2 (disrupted) cells became not only cs but also temperature sensitive.

The relative sensitivity to TBZ of the parental diploid SP286 and *Δnda2* (*nda2$^+$/nda2$^-$*) mutant was investigated, revealing the TBZ-induced haploinsufficient phenotype of *Δnda2* (*nda2$^+$/nda2$^-$*) (Fig. 2). When thirteen heterozygous deletion mutants were tested for their sensitivity to TBZ, only *Δnda2* (*nda2$^+$/nda2$^-$*) showed a distinct sensitivity to

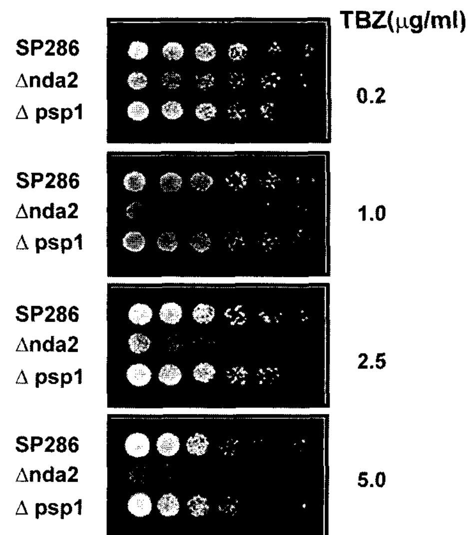

Fig. 2. TBZ sensitivity of *Δnda2* (*nda2$^+$/nda2$^-$*) mutant.
The exponentially growing parental diploid SP286 and heterozygous diploid *Δnda2* (*nda2$^+$/nda2$^-$*) and *Δpsp1*(*psp1$^+$/psp1$^-$*) were diluted to 2×10^7 cells/ml in 3-fold serial dilutions and 5 μl of cells were spotted onto a minimal plate containing thiabendazole (0.2, 0.5, 1.0, 5.0 μg/ml) and incubated for 2-3 days.

TBZ (Fig. 3), indicating a TBZ-induced haploinsufficient phenotype. As such, this result suggests that profiling drug-induced haploinsufficiency can be exploited to identify target genes for drugs.

To test the feasibility of molecular tags to monitor the population of *Δnda2* mutants in a pooled assay, equal numbers of thirteen different heterozygous deletion mutants were pooled and grown in the absence and presence of

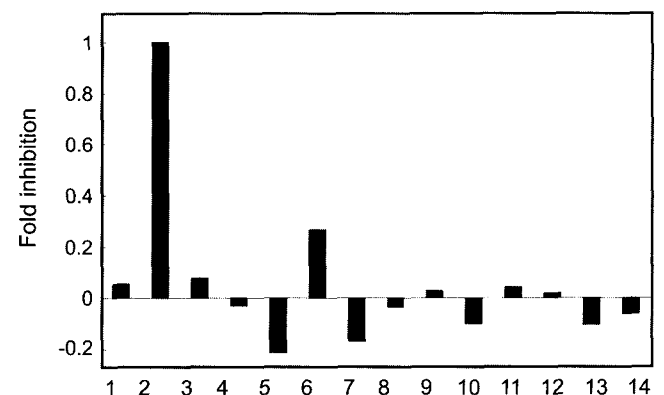

Fig. 3. Cytotoxicity of TBZ.
Exponentially growing cells of the heterozygous diploid mutants were diluted to 1.5×10^5 cells/ml and dispensed into 96-well plates. TBZ was dispensed in five 2-fold serial dilutions with the highest concentration at 50 μg/ml. The plates were incubated for 18 h at 30°C and the A_{600} of the cultures was read in a VERSAmax microplate reader, using SOFTmax PRO. The relative cytotoxicity of TBZ was analyzed. 1, SP286; 2, *Δnda2*; 3, *Δshk1*; 4, *Δcrm1*; 5, *Δste7*; 6, *Δste11*; 7, *Δdis3*; 8, *Δdis2*; 9, *Δrad16*; 10, *Δrad24*; 11, *Δchk1*; 12, *Δcds1*; 13, *Δcdr1*; 14, *Δcdc25*.

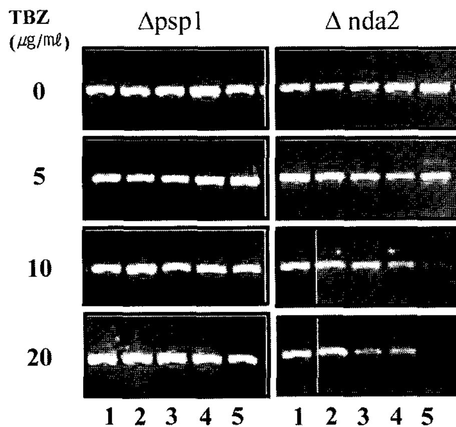

Fig. 4. Haploinsufficiency of *Δnda2* (*nda2*$^{+}$/*nda2*$^{-}$) in the pooled assay

Equal numbers of 18 heterozygous deletion mutants were pooled and grown in the absence and presence of TBZ (0, 5.0, 10.0, 20.0 μg/ml) for 50 h. The existence of the *Δnda2*(*nda2*$^{+}$/*nda2*$^{-}$) and *Δpsp1*(*psp1*$^{+}$/*psp1*$^{-}$) mutants was examined by a PCR using gene-specific primers. 1, 0 h; 2, 10 h; 3, 24 h; 4, 34 h; 5, 50 h.

thiabendazole (0, 5.0, 10.0, 20.0 μg/ml) for 50 h. The existence of *Δnda2*(*nda2*$^{+}$/*nda2*$^{-}$) and *Δpsp1*(*psp1*$^{+}$/*psp1*$^{-}$) mutants in the culture was examined by a PCR using gene-specific primers. As shown in Fig. 4, no *nda2*-specific PCR product was detected in the cells grown for 50 h in the presence of TBZ, indicating that *Δnda2*(*nda2*$^{+}$/*nda2*$^{-}$) had completely disappeared. Therefore, drug-induced haploinsufficiency can be a powerful tool for identifying drug targets, using a high density oligonucleotide microarray system.

Thousands of deletion mutants of *S. pombe* can be exploited to determine the biological functions of newly identified yeast and human genes by parallel analysis and functional complementation. Since the yeast cell-based assay system for screening is useful [5], the pool of deletion mutants can provide a powerful tool for the rapid and cost-effective identification of drug target and functional analysis of genes.

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