Cross-Species Complementation of Nonessential Yeast Genes Establishes Platforms for Testing Inhibitors of Human Proteins

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ABSTRACT Cross-species complementation can be used to generate humanized yeast, which is a valuable resource with which to model and study human biology. Humanized yeast can be used as an in vivo platform to screen for chemical inhibition of human protein drug targets. To this end, we report the systematic complementation of nonessential yeast genes implicated in chromosome instability (CIN) with their human homologs. We identified 20 human–yeast complementation pairs that are replaceable in 44 assays that test rescue of chemical sensitivity and/or CIN defects. We selected a human–yeast pair (hFEN1/yRAD27), which is frequently overexpressed in cancer and is an anticancer therapeutic target, to perform in vivo inhibitor assays using a humanized yeast cell-based platform. In agreement with published in vitro assays, we demonstrate that HU-based PTPD is a species-specific hFEN1 inhibitor. In contrast, another reported hFEN1 inhibitor, the arylstibonic acid derivative NSC-13755, was determined to have off-target effects resulting in a synthetic lethal phenotype with yRAD27-deficient strains. Our study expands the list of human–yeast complementation pairs to nonessential genes by defining novel cell-based assays that can be utilized as a broad resource to study human drug targets.

KEYWORDS human–yeast cross-species complementation; chromosome instability; chemical inhibitors; FEN1; cancer targets

OFF-TARGET effects are a major cause of clinical trial failures for cancer therapeutics (Lin et al. 2019). As such, establishing additional preclinical models can contribute to the translation of more effective clinical outcomes. One such model is the humanized yeast system, which has been used as an in vivo platform for studying chemical inhibition of human protein targets [reviewed in Simon and Bedalov (2004), Mager and Winderickx (2005), and Zimmermann et al. (2018)]. Yeast can be humanized using two different approaches: heterologous expression in which a human gene is expressed ectopically in yeast or cross-species complementation in which the human gene complements a mutation in the cognate yeast gene [reviewed in Dunham and Fowler (2013) and Laurent et al. (2016)]. Irrespective of orthology, heterologous expression of human genes that induce a phenotypic readout in wild-type yeast cells (such as growth inhibition) can be leveraged to elucidate the pathological functions of disease genes (Cooper et al. 2006), identify drug targets (Jo et al. 2017), and screen for chemical inhibitors that rescue the growth defect (Perkins et al. 2001; Tugendreich et al. 2001; Sekigawa et al. 2010). In cases where a yeast homolog can be identified for a human gene, cross-species complementation of yeast mutations by human genes can be utilized to elucidate the functional homology between human and yeast proteins (Lee and Nurse 1987), characterize human disease variants (Marini et al. 2008; Trevisson et al. 2009; Mayfield et al. 2012; Sun et al. 2016; Yang et al. 2017), evaluate tumor-specific mutations (Shaag et al. 2005; Hamza et al. 2015), and screen for chemical inhibitors (Marjanovic et al. 2010). Several large-scale studies have systematically tested the ability of single human genes to replace their yeast orthologs (Zhang et al. 2003; Hamza et al. 2015; Kachroo et al. 2015; Sun et al. 2016) and paralogs (Hamza et al. 2015; Yang et al. 2017; Garge et al. 2019; Laurent et al. 2019). However, the focus of these complementation screens was restricted to essential yeast
genes whose mutation allowed for testing the rescue of lethal growth defects. In contrast, nonessential yeast genes, the majority of which cause minimal growth defects when disrupted, can only be screened for complementation of visible phenotypes or in conditional assays that induce measurable growth phenotypes. Conditional assays could involve growing the nonessential gene mutants in restrictive media conditions, e.g., alternate sugar sources (Guimier et al. 2016) or a limiting metabolite (Agmon et al. 2019), adding chemicals to sensitize the yeast strain, or converting the nonessential yeast gene to an essential gene by disrupting a synthetic lethal partner (Greene et al. 1999).

Chromosome instability (CIN) mutants are of particular interest for human complementation in yeast. CIN is an enabling characteristic of cancer development and progression, and is a major contributor to the heterogeneity of tumors (Negrini et al. 2010; Hanahan and Weinberg 2011). The simplicity and genetic tractability of the budding yeast, *Saccharomyces cerevisiae*, make it a model experimental system to delineate conserved biological pathways and processes such as those involved in CIN (Measday and Stirling 2016). Large-scale yeast screens have generated a comprehensive list of genes whose mutation (Myung et al. 2001; Smith et al. 2004; Kanelis et al. 2007; Yuen et al. 2007; Andersen et al. 2008; Stirling et al. 2011) or overexpression (Zhu et al. 2015; Ang et al. 2016; Duffy et al. 2016; Frumkin et al. 2016; Tutut et al. 2019) contribute to CIN. Yeast can also be utilized to identify chemical sensitivities to cytotoxic agents caused by CIN gene mutations that may be exploited to selectively target tumor cells (O’Neil et al. 2017). For instance, genotoxic agents that act by alkylation are common cancer chemotherapcy drugs and yeast mutants that are sensitive to these agents identify candidate human genes required for the DNA damage response (Svensson et al. 2012).

Proteins required for chromosome stability are also attractive targets for therapeutic inhibition in cancer cells (Tanaka and Hirota 2016). Indeed, the yeast CIN gene list identifies candidate human CIN genes whose mutation or overexpression may contribute to tumorigenesis (Barber et al. 2008; Stirling et al. 2011; Duffy et al. 2016). One such attractive target is the human DNA flap endonuclease 1 (hFEN1). Yeast assays have demonstrated that deletion or overexpression of yRAD27 (ortholog of hFEN1) causes CIN and DNA damage in yeast (Greene et al. 1999; Yuen et al. 2007; Duffy et al. 2016), while studies using human cells have confirmed that deletion or overexpression of hFEN1 causes DNA damage (Jimeno et al. 2017; Becker et al. 2018; Mengwasser et al. 2019). FEN1 functions in DNA replication and repair, and is required for Okazaki fragment maturation through removal of 5’ flaps during lagging-strand synthesis (Balakrishnan and Bambara 2013). Due to its key role in DNA replication, hFEN1 has been shown to support rapid proliferation of cancer cells and is overexpressed in breast (Singh et al. 2008; Abdel-Fatah et al. 2014; He et al. 2016), lung (Nikolova et al. 2009; He et al. 2017), prostate (Lam et al. 2006), gastric (Wang et al. 2014), brain (Krause et al. 2005), and pancreatic (Jacobuzio-Donahue et al. 2003) cancer. Further, based on synthetic lethal genetic interaction relationships, hFEN1 is a second-site target for the selective killing of homologous recombination (HR)-defective and cohesin-mutated cancer cells (McManus et al. 2009; van Pel et al. 2013). As such, many studies have reported the screening and development of hFEN1 inhibitors as potential anticancer therapeutics (Tumey et al. 2005; Dorjsuren et al. 2011; McWhirter et al. 2013; van Pel et al. 2013; Exell et al. 2016; He et al. 2016; Deshmukh et al. 2017).

A humanized yeast system based on cross-species complementation can be utilized as an in vivo platform for inhibitor screening. Human complementation of essential yeast gene mutants has been used as a phenotypic readout to screen for inhibitors of human proteins that induce growth inhibition in yeast (Meczys et al. 1997; Wider et al. 2009; Marjanovic et al. 2010; Mayi et al. 2015). To expand the list of yeast cell-based platforms beyond those discovered for essential yeast genes, we screened a subset of 112 nonessential yeast CIN genes for complementation by candidate human homologs. We then demonstrated that complementation of nonessential yeast genes in conditional assays that induce measurable growth phenotypes is a viable strategy to test inhibitors of hFEN1.

**Materials and Methods**

**Complementation assays**

*Generating expression vectors and yeast strains for complementation assays*: A list of human homologs of nonessential yeast CIN genes (Yuen et al. 2007; Stirling et al. 2011) was generated from Yeastmine (Balakrishnan et al. 2012). Human complementary DNAs (cDNAs) in Gateway-compatible entry clones (Yang et al. 2011) were shuttled into the yeast destination vector pAG416GPD-ccdB+6Stop (*URA3*, CEN, constitutive GPD promoter, and 6-amino acid C-terminal extension) (Alberti et al. 2007; Kachroo et al. 2015) using LR Clonase II (Invitrogen, Carlsbad, CA) to generate expression clones. Expression vectors and the vector control pRS416 (*URA3*) (Sikorski and Hieter 1989) were transformed into the corresponding *MATα* yeast haploid knockout strain (Giaever et al. 2002), and wild-type strain *MATα* BY4742 (Brachmann et al. 1998) and transformants were selected on SC-Ura media.

To create yeast strains with integrated human cDNAs, donor DNA generated by PCR was cotransformed into BY4742 along with linear fragments encoding Cas9 and a single guide RNA targeted to the coding region of either *yRAD1*, *yRAD10*, *yMMS4*, or *yMUS81* (Supplemental Material, Table S1), following the protocol described by William Shaw (see https://benchling.com/pub/ellis-crispr-tools). Donor DNA for *hMUS81* was obtained by PCR using the entry clone from hORFeome V8.1 (Yang et al. 2011) as template and primers were designed to include a stop codon. Donor DNA for *hERCC4* and *hME1* was obtained by PCR using clones from the Mammalian Gene Collection (Dharmacon) as template. Donor DNA for *hERCC1* was generated using
pAG416GPD-hERCC1 +6Stop as template in the PCR resulting in a PCR product that also contained the CYC1 terminator. The double-deletion strains mus81Δ mms4Δ and rad1Δ rad10Δ were made by clustered regularly interspaced short palindromic repeats (CRISPR)-mediated deletion (Table S1) of MMS4 and RAD10 in the mus81Δ::kanMX and rad1Δ::kanMX MATα deletion strains, respectively. For the deletions, donor DNA was constructed by annealing two complimentary oligos composed of flanking homology to the left and right of the deletion site. To confirm CRISPR-mediated insertions or deletions, yeast transformants were screened by PCR using primers that flank the region of homology on the donor DNA and verified by sequencing.

Sequence identity (%) in relation to the yeast gene was determined for all human–yeast pairs using NWalign (Y. Zhang, http://zhanglab.ccmb.med.umich.edu/NW-align), and significance between the % sequence identity of complementation pairs and % sequence identity of tested human–yeast pairs was calculated using a Mann–Whitney U-test. Yeast gene size was obtained from Yeastmine and significant differences were calculated using the hypergeometric distribution.

**Growth assays to assess rescue of chemical sensitivities:** Chemical sensitivity complementation assays for yeast strains with URA3-marked vectors were carried out in SC-Ura media (± chemical) at 30°, while assays for yeast strains with integrated human cDNAs were carried out in SC media (± chemical) at 30°. For spot assays, wild-type and mutant strains from saturated cultures were serially diluted in 10-fold increments, and plated onto media without or with chemicals at the following concentrations: 0.01% MMS, 200 mM HU, 15 μg/ml benomyl, 8% ethanol, 100 ng/ml cycloheximide, 5 μg/ml camptothecin (CPT), and 10 μg/ml bleomycin. For growth curve validations, yeast strains from midlog phase cultures were grown to midlog phase then diluted to OD600 = 0.1 in 200 μl media ± chemical at the indicated concentrations. OD600 readings were measured every 30 min over a period of 24–48 hr in a TECAN M200 plate reader and plates were shaken for 10 min before each reading. Strains were tested in three replicates per plate per condition and the AUC was calculated for each replicate. Strain fitness was defined as the AUC of each yeast strain relative to the AUC of the control strain (pdr1Δ pdr3Δ + 0.5% DMSO control) grown on the same plate ± MMS treatment.

**Overexpression of hFEN1:** Human FEN1 in an entry clone was shuttled into the yeast destination vector pAG425GAL-ccdB+6Stop (LEU2, 2μ, inducible GAL promoter, and 6-amino acid C-terminal extension) (Alberti et al. 2007; Kachroo et al. 2015) as previously described in the Materials and Methods. The overexpression vector and the vector control pRS425 (Christianson et al. 1992) were transformed into BY4742, and transformants were selected on SC-Leu media. Generated strains were grown to midlog phase in both dextrose or galactose SC-Leu media before dilution to OD600 = 0.1 in the same media for growth curve analysis as previously described in the Materials and Methods.

**Data availability**

Strains and plasmids are available upon request. Figure S1 contains complementation assays for the nonessential CIN genes. Figure S2 contains complementation assays of two-subunit yeast complexes. Figure S3 contains growth curve assays for testing hFEN1 inhibitors in MMS. Figure S4 contains growth curve assays for testing hFEN1 inhibitors in HR-deficient strains. Figure S5 contains growth curve assays assessing overexpression of hFEN1 in yeast. Table S1 lists primers used for CRISPR-mediated insertion and deletion. Table S2 lists plasmids and strains used in study. Table S3 lists all candidate complementation pairs tested in study.
Table S4 compares our list of complementation pairs to literature sources. Supplemental material available at figshare: https://doi.org/10.25386/genetics.11573229.

Results

Identification of human–yeast complementation pairs for the nonessential yeast CIN genes

A comprehensive list of yeast CIN genes revealed that of the \(~1100\) essential yeast genes, \(323\) are mutable to a CIN phenotype (Stirling et al. 2011). Likewise, of the \(~4800\) nonessential yeast genes, \(369\) are mutable to a CIN phenotype as deletion alleles (Yuen et al. 2007; Stirling et al. 2011). Previously, we screened the essential yeast CIN genes for complementation by corresponding human cDNAs by assaying rescue-of-lethality of the haploid yeast null allele derived from sporulation of heterozygous diploid strains (Hamza et al. 2015). In this study, we identified human–yeast complementation pairs for the nonessential yeast CIN genes. In contrast to essential genes, most haploid deletion strains for nonessential genes display no growth defects when grown under standard laboratory conditions (Giaever et al. 2002). To establish our complementation assays, we tested the ability of human gene expression to rescue the chemical sensitivity and/or CIN defects of the yeast knockout strain. The chemicals utilized to induce growth defects included MMS (alkylating agent) (Beranek 1990), benomyl (destabilizes microtubules) (Gupta et al. 2004), HU (impedes DNA replication) (Koç et al. 2004), CPT (topoisomerase inhibitor) (Hsiang et al. 1989), bleomycin (induces DNA strand breaks) (Chen et al. 2008), cycloheximide (protein synthesis inhibitor) (Schneider-Poetsch et al. 2010), and ethanol (impacts many cellular pathways including the cell cycle and morphogenesis) (Stanley et al. 2010). To test rescue of CIN defects, we...
used the ALF assay, which measures loss of the MATα locus leading to dedifferentiation to an a-mating phenotype and subsequent mating to a MATα tester strain (Yuen et al. 2007; Stirling et al. 2011). In this assay, the ability of haploid cells to mate with a tester strain of the same mating type and form diploids reflects loss, deletion, or inactivation of the MATα locus.

To set up the complementation assays, we generated a list of human sequence homologs of nonessential yeast CIN genes using the Yeastmine database (Balakrishnan et al. 2012) (Figure 1A). This included orthologs, which are genes that diverged by speciation and typically perform the same biological function across species, and paralogs, which diverged by duplication and generally perform biologically distinct yet mechanistically related functions (Koonin 2005). Each human ORF was shuttled via Gateway cloning into a yeast expression vector (single-copy centromeric plasmid and constitutive GPD promoter) (Alberti et al. 2007; Kachroo et al. 2015) (Figure 1B). For each yeast strain, we queried the Saccharomyces Genome Database (SGD) to determine if the yeast deletion mutant has fitness defects in the presence of at least one of the indicated chemicals. We also searched previously published reports (Yuen et al. 2007; Stirling et al. 2011) to identify mutants that display increased diploid mating products in ALF assays compared to the wild-type strain.

Overall, this established assayable phenotypes to test 112 nonessential yeast CIN deletion mutants for complementation by 117 human cDNAs (121 candidate complementation pairs tested across 317 complementation assays) (Figure 1C and Table S3). Our screens identified 20 human cDNAs that rescue the chemical sensitivity and/or CIN defects of 20 nonessential yeast mutants (Table 1). Successful complementation pairs were validated by growth curves and encompassed 44 assays that ranged from one to four assays per pair (Figures S1 and S2). For instance, we demonstrated that hTBCC expression rescues cin2Δ sensitivity to benomyl (Figure 1D), and that hFEN1 expression rescues rad27Δ sensitivity to MMS, ethanol, and cycloheximide, as well as rescuing CIN defects of rad27Δ strains in the ALF assay (Figure S1).

Based on a curated list of complementation pairs available from the Yeastmine database, our work identified 13 novel complementation pairs (Table S4).

We assessed features that predict complementation of nonessential yeast gene deletion mutants. Previous screens that tested complementation of essential yeast genes determined that the strongest predictive feature of complementation is that genes in the same pathway, process, or complex tended to be similarly replaceable or nonreplaceable (Hamza et al. 2015; Kachroo et al. 2015, 2017; Sun et al. 2016).

Table 1 Human genes that complement nonessential yeast deletion mutants

| Yeast systematic name | Yeast standard name | Human Entrez gene identifier | Human standard name | Complementation assay* | Yeast gene brief descriptionb |
|-----------------------|---------------------|-----------------------------|---------------------|------------------------|--------------------------------|
| YAL016W               | TPD3                | 5518                        | PPP2R1A             | MMS, HU, ALF           | Regulatory subunit A of the heterotrimeric PP2A complex |
| YBR026C               | ETR1                | 51102                       | MECHR               | Ethanol, cycloheximide  | 2-enzoyl thioester reductase |
| YDR226W               | ADK1                | 204                         | AK2                 | Ethanol                | Adenylate kinase, required for purine metabolism |
| YDR363W-A             | SEM1                | 7979                        | SHFM1               | HU, ethanol, ALF        | 19S proteasome regulatory particle lid subcomplex component |
| YEL003W               | GIM4                | 5202                        | PFDN2               | Benzymyl, ethanol       | Subunit of the heterohexameric cochaperone prefoldin complex |
| YEL029C               | BUD16               | 8566                        | PDKX                | Ethanol                | Putative pyridoxin kinase |
| YGL058W               | RAD6                | 7320                        | UBE2B               | MMS, HU, bleomycin, ALF | Ubiquitin-conjugating enzyme (E2) |
| YGR078C               | PAC10               | 7411                        | VBP1                | Ethanol, cycloheximide, ALF | Part of the heterohemeric cochaperone GmC/ prefoldin complex |
| YGR180C               | RNR4                | 6241                        | RRM2                | MMS, HU, bleomycin     | Ribonucleotide-diphosphate reductase (RNR) small subunit |
| YIL052C               | RPL34B              | 6164                        | RPL34               | Ethanol                | Ribosomal 60S subunit protein L34B |
| YIL115W               | ASF1                | 55723                       | ASF1B               | MMS, CPT, ALF          | Nucleosome assembly factor |
| YIL140W               | RP84                | 5433                        | POL2RD              | HU, MMS                | RNA polymerase II subunit B2 |
| YKL113C               | RAD27               | 2237                        | FEN1                | MMS, ethanol, cycloheximide, ALF | 5’ to 3’ exonuclease, 5’ flap endonuclease |
| YLR418C               | CDC73               | 79577                       | CDC73               | HU                     | Component of the Paflp complex |
| YML094W               | GIM5                | 5204                        | PFDN5               | Ethanol, cycloheximide  | Subunit of the heterohemeric cochaperone prefoldin complex |
| YML095C               | RAD10               | 2067                        | ERCC1               | MMS, HU                | Single-stranded DNA endonuclease (with Rad1p) |
| YOL012C               | HTZ1                | 3015                        | H2AFZ               | MMS, HU, ethanol        | Histone variant H2AZ |
| YOR002W               | ALG6                | 29929                       | ALG6                | Ethanol                | α 1, 3 glucosyltrasferase |
| YPL022W               | RAD1                | 2072                        | ERC4                | MMS, HU                | Single-stranded DNA endonuclease (with Rad10p) |
| YPL241C               | CIN2                | 6903                        | TBCC                | Benzymyl               | GTPase-activating protein (GAP) for Cin4p |

* Complementation assays are shown in Figure S1.

b Brief description obtained from Yeastmine.
Human genes were more likely to complement if they had a higher proportion of interacting partners that also complemented and vice versa. We observed that this property also applies to the nonessential yeast genes. For example, we determined that each member of the yRad1/yRad10 endonuclease complex was replaceable by their respective human orthologs, hERCC4/hERCC1, individually. Conversely, for another endonuclease complex composed of yMUS81/yMMS4, neither subunit was replaceable by either hMUS81 or hEME1 individually. To examine if both members of the complex were required for a successful complementation, we used CRISPR/Cas9-based genomic engineering to replace both members of the yeast complex with the human orthologs. This generated yeast strains in which the human gene ORFs are integrated in the genome and under control of the native yeast gene regulation. A CRISPR/Cas9-mediated ORF replacement strategy ensures stable and endogenously regulated human gene expression that accounts for stoichiometric balance of complex subunits (Ryan et al. 2014), while addressing the limitations on the number of yeast selection markers needed for inactivation of multiple yeast genes and simultaneous expression of multiple human genes in yeast. Unlike hERCC4/hERCC1, which could be successfully replaced as a complex (Figure 2A), the combined hMUS81/hEME1 complex failed to complement its yeast heterodimer counterpart (Figure 2B). Other prominent features that were determined to be in common between replaceable essential and nonessential yeast genes are yeast gene size and human/yeast protein sequence similarity. Replaceable nonessential yeast genes were more likely to be shorter in length (Figure 3A), while human/yeast protein sequence identity was found to be a poor predictor of replaceability (Figure 3B). Even though complementation pairs tend to have higher than average sequence similarity, sequence features alone cannot predict a successful complementation. Overall, our results indicate that features that predict complementation of nonessential yeast genes are similar to those observed for essential yeast genes.

**Cross-species complementation as a platform for testing hFEN1 inhibitors**

Our human–yeast complementation screen demonstrated that hFEN1 can replace yRad27, thereby presenting an in vivo yeast cell-based platform to test chemical inhibition of the human protein. We selected two commercially available compounds reported to inhibit enzymatic activity of hFEN1 in vitro to test in our complementation assays: the N-hydroxyurea-based PTPD and the arylstibonic acid derivative NSC-13755 (Dorjsuren et al. 2011). To increase membrane permeability, CRISPR/Cas9-based genomic engineering was utilized to construct yeast strains deficient in yPdr1 and yPdr3, which regulate the multidrug resistance pathway (Balzi and Goffeau 1995). Rather than expressing hFEN1 from a plasmid with the GPD promoter, the CRISPR system was used to replace the genomic yRad27 ORF with the hFEN1 ORF so that hFEN1 was expressed from the yRad27 promoter.

In the absence of added hFEN1 inhibitor, the yeast rad27Δ null mutant displays a near wild-type growth rate but is sensitive to treatment with 0.01% MMS (Figure 4, black bars). Expression of hFEN1 (rad27Δ::hFEN1) fully rescues the MMS sensitivity (Figure 4). We tested the ability of the two candidate hFEN1 inhibitors to cause growth inhibition of the rad27Δ::hFEN1 yeast strain in the presence of MMS. PTPD elicted concentration-dependent inhibition of hFEN1 as measured by fitness of the rad27Δ::hFEN1 yeast

![Figure 2](Image)

**Figure 2** Testing complementation of two-subunit yeast complexes. (A) hERCC4/hERCC1 expression (separately or together) rescues rad1Δ/rad10Δ sensitivity to MMS (0.01%) and HU (150 mM). Student’s t-test. ***P < 0.01; ****P < 0.0001. (B) hMUS81/hEME1 expression (separately or together) does not rescue mus81Δ/mms4Δ sensitivity to MMS and HU. Each strain was tested in three replicates per condition and the AUC value was calculated for each replicate independently. Strain fitness was defined as the AUC of each yeast strain relative to the AUC of the wild-type (BY4742) strain grown in the same media condition (mean ± SD). Corresponding growth curves are shown in Figure S2. AUC, area under the curve.
by CRISPR-mediated deletion of y in the rad52 strain did not impact that PTPD inhibits hFEN1 to con-hibit synthetic lethality with the same extent by either y severe defects in the rad27 mutant, which may indicate inhibition of additional off-target yeast protein(s) resulting in negative genetic interactions that impact rad27 strain fitness. In contrast, NSC-13755 elicited phenotypes inconsistent with hFEN1 inhibition. NSC-13755 did not elicit concentration-dependent inhibition of growth as measured by fitness of the rad27Δ::hFEN1 yeast strain in MMS (Figure 4B). At the highest concentration of NSC-13755 tested (1 mM), rescue of rad27Δ sensitivity to MMS was observed to the same extent by either yRAD27 or hFEN1. Furthermore, in the absence of MMS, NSC-13755 caused severe fitness defects in the rad27 mutant (at the highest concentration tested, 1 mM), and these defects were rescued to the same extent by either yRAD27 or hFEN1, indicating NSC-13755 inhibition of off-target yeast protein(s) that exhibit synthetic lethality with the rad27Δ mutant (Figure 4B). To confirm these results, we generated HR-deficient strains by CRISPR-mediated deletion of yRAD52 in yRAD27 and rad27Δ::hFEN1 yeast strains. Given that the yeast rad27Δ rad52Δ double mutant shows a synthetic lethal phenotype (Symington 1998), hFEN1 inhibition can be assessed by in-hibition of growth in the absence of MMS. While PTPD caused mild fitness defects in the yRAD27 rad52Δ strain, PTPD caused severe concentration-dependent growth defects in the rad27Δ::hFEN1 rad52Δ strain (Figure 5A), confirming that PTPD inhibits hFEN1 in vivo. In contrast, NSC-13755 did not impact fitness of the rad27Δ::hFEN1 rad52Δ strain relative to the yRAD27 rad52Δ strain (Figure 5B). Notably, the differential sensitivity of the rad27Δ mutant (relative to yRAD27 wild-type) caused by NSC-13755 (Figure 4B) was not observed for the rad52Δ mutant (relative to yRAD52 wild-type), suggesting that the off-target effects of this compound may be restricted to yeast protein(s) that are synthetic lethal with the rad27Δ null allele. Overall, these results demon-strate the utility and feasibility of using complementation in conditional assays to test chemical inhibition of human protein targets in a cell-based platform.

**Discussion**

The development of high-throughput and large-scale technologies have expanded the screening capacity for human–yeast complementation pairs. As a result, several systematic screens have reported testing the essential yeast genes for replaceability (Zhang et al. 2003; Hamza et al. 2015; Kachroo et al. 2015; Sun et al. 2016; Yang et al. 2017; Garge et al. 2019; Laurent et al. 2019). These studies generated overlapping lists of human–yeast complementation pairs and arrived at similar conclusions regarding features that predict the replaceability of essential yeast genes. However, compared to the essential yeast genes, the nonessential genes are a much larger set and have a variety of different phenotypic readouts, making them more difficult to screen systematically for complementation. In this study, we have started this process by focusing on a subset of the nonessential yeast genes, specifically those required for chromosome maintenance. We identified 20 complementation pairs that are replaceable in 44 assays that test rescue of chemical sensitivity and/or CIN defects. For some human–yeast pairs, we demonstrated that the human gene can complement the yeast gene in multiple complementation assays. Although we did not identify any in this study, there are reported cases of complementation pairs that complement some but not all mutant phenotypes (Yamagata et al. 1998; Tamburini et al. 2005; Davey et al. 2011). For instance, hWRN (homolog of ySGS1) suppressed the increased rate of illegitimate recombination of sgs1Δ but could not rescue sgs1Δ sensitivity to HU (Yamagata et al. 1998). Our study defines 44 complementation assays that provide yeast cell-based platforms to elucidate human protein function, characterize human gene variants, and study conserved protein domains based on human–yeast complementation relationships. The utility of these yeast cell-based platforms (Dunham and Fowler 2013; Laurent et al. 2016) and the accelerated pace of discovery for human–yeast complementation pairs has led the SGD to curate complementation data in their Yeastmine database (Balakrishnan et al. 2012).

Our analysis of complementing/noncomplementing human–yeast gene pairs found that features predicting the replaceability of nonessential yeast genes were similar to those observed for essential yeast genes. In general, a major limitation to cross-species complementation involving multiprotein complexes is the potential for interactions of the human protein subunits with the cognate yeast interaction partners. We presented the example of humanizing two related endonucleases that each form heterodimers and function in similar DNA repair processes with some functional overlap (Kikuchi et al. 2005; Davey et al. 2011). In one case, each subunit of the yRad1/yRad10 endonuclease was replaceable, indicating that

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**Figure 3** Analyzing features of nonessential yeast genes that predict replaceability. (A) Replaceable nonessential yeast genes are more likely to be shorter in length. Yeast genes were binned by gene length (base pair) and represented as a proportion of the total number of genes input for each set. (B) Amino acid sequence identity is not a strong predictive feature of complementation. The box plot highlights the medians and ranges of sequence identity for each set of human–yeast gene pairs.

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| Yeast Gene Size (bp) | % Yeast Genes | Replaceable Genes tested (n=112) | Nonessential Genes tested (n=20) |
|----------------------|--------------|---------------------------------|---------------------------------|
| <1250                | 50           | 50                              | 50                              |
| >1250                | 50           | 50                              | 50                              |

| Gene pairs tested (n=121) | Replaceable gene pairs (n=20) |
|---------------------------|-------------------------------|
|                           |                               |
each human ortholog was able to form a heterodimer with the other yeast subunit. In contrast, yMus81/yMms4 was nonreplaceable even when combining both human subunits in the yeast strain. While these results are consistent with previous findings that showed that genes in the same complex tend to be similarly replaceable or nonreplaceable (Hamza et al. 2015; Kachroo et al. 2015, 2017; Sun et al. 2016), there are reported cases of two-subunit yeast complexes that are only replaceable when both human protein orthologs are expressed (Katahira et al. 1999; Gao et al. 2005; Ozanick et al. 2005; Arnesen et al. 2009; Davey et al. 2011; Paul et al. 2015). In another study, the four-subunit yeast nucleosome was shown to be replaceable by the human nucleosome only after a rare event that allowed yeast cells to adapt and acquire suppressor mutations (Truong and Boeke 2017). A more robust complementation was observed after converting five human histone amino acid residues to the amino acids found in their yeast counterparts, thus revealing the importance of interactions in the success of a human complementation experiment. Overall, our results suggest that the major limitation to predicting the ability of some yeast complexes to be humanized is the lack of information on the minimum complex/pathway members that need to be replaced simultaneously. While replacing one subunit of yRad1/yRad10 was sufficient for complementation, humanization of yMus81/yMms4 may require the replacement of additional yeast genes by human proteins to regulate the functions of the humanized endonuclease (Déhé and Gaillard 2017). Thus, attempts to discover and test the minimum requirements for humanization can provide new avenues to study important properties of human proteins.

We demonstrated an application of humanized yeast in which cross-species complementation of chemical sensitivity facilitated testing candidate inhibitors of hFEN1. Generally, there are two main approaches to screen for inhibitors of human targets in a humanized yeast system: (i) inhibitors that rescue yeast growth defects caused by ectopic heterologous expression of the human gene in yeast and (ii) inhibitors that are screened against the ability of human gene expression to rescue growth defects of the yeast mutant. As a platform for inhibitor screening, human complementation of the yeast null mutant has several benefits over ectopic heterologous expression of human genes in yeast. Human proteins that complement yeast mutants are functional in a cellular system and in the context of other biological pathways, and inhibitors that prevent complementation may better reflect inhibition of the human protein activity in a cellular context. Screening in a null mutant background further eliminates the potential of non-specific inhibition of the cognate yeast protein and further facilitates the identification of inhibitors that are species-specific (Wider et al. 2009; Bilsland et al. 2016). Moreover, severe growth defects caused by ectopic heterologous expression of human genes in yeast may be reversed by suppressor mutations that bypass the fitness defects and, as such, large-scale screens for inhibitors that rescue growth defects may have high backgrounds due to false positives. Cross-species complementation may also provide an alternative phenotypic readout for human proteins that do not induce severe toxicity in yeast. For example, overexpression of hFEN1 in wild-type yeast causes a minimal growth defect (~15%) (Figure S5), which is not enough of a differential sensitivity for a
Figure 5 Utilizing complementation to test hFEN1 inhibitors in homologous recombination-deficient yeast. (A) PTPD reduces fitness of a hFEN1-integrated strain deficient in yRAD52. (B) NSC-13755 does not impact fitness of yRAD52-deficient yeast. All strains in this figure are in a pdr1Δ pdr3Δ background. Each strain was tested in three replicates per condition and the AUC value was calculated for each replicate independently. Strain fitness was defined as the AUC of each yeast strain relative to the AUC of the control strain (pdr1Δ pdr3Δ + 0.5% DMSO) grown in the same media condition (mean ± SD). The control strains presented in this figure are the same as in Figure 4 since the experiments were performed in the same plate. "No MMS" refers to SC media. Corresponding growth curves are shown in Figure S4. Student’s t-test. **** P < 0.0001. AUC, area under the curve.

Potential Development of Human Gene Complementing Yeast Cells as a Surrogate System

Our results underscore the necessity of coupling in vitro enzymatic assays with in vivo assays when assessing inhibitor target activity and specificity. The in vitro assays identified PTPD as a more potent inhibitor of hFEN1 than NSC-13755 (Dorjsuren et al. 2011). This result may be reflected in our in vivo growth assays that determined that NSC-13755 is not potent enough to inhibit hFEN1 activity to a level that causes growth inhibition comparable to the rad27Δ mutant. Instead, NSC-13755 seemed to be a potent inhibitor of other yeast protein(s) that are synthetic lethal with the rad27Δ mutant. Consistent with this observation, in vitro assays have classified NSC-13755 and other arylostibonic acid derivatives as enzymatic inhibitors of the human apurinic/apyrimidinic endonuclease hAPE1 (Seiple et al. 2008; Simeonov et al. 2009), the regulatory phosphatases hPTEN and hCDC25 (Mak et al. 2012), and topoisomerase IB (hTOP1) (Kim et al. 2008), in addition to hFEN1. Based on the in vivo assays presented in our study, we cannot deduce which yeast homolog(s) of these human protein in vitro targets, or other potential yeast protein(s), are the in vivo target(s) of NSC-13755. However, a humanized yeast system in which ectopic expression of these human proteins in yeast results in measurable phenotypic readouts may prove to be a suitable in vivo platform to determine the target most sensitive to the inhibitory activity of NSC-13755. The effectiveness of such a system is reflected in our in vivo assays for the HU-based PTPD. While this inhibitor did display some mild off-target effects, it appeared to be a potent hFEN1 inhibitor. In support of our results, other HU-based derivatives have been shown to increase human cell line sensitivity to MMS treatment (Tumey et al. 2005; Exell et al. 2016) and selectively impair the proliferation of HR-defective cancer cell lines (Exell et al. 2016; He et al. 2016; Ward et al. 2017). Further, in vitro assays have demonstrated that some HU-based compounds are able to enzymatically inhibit the related endonucleases hXPG (Tumey et al. 2005) and hEXO1 (Exell et al. 2016), but showed species specificity upon testing with bacteriophage T5 FEN and Klyveromyces lactis XRN1 (Exell et al. 2016). These results may explain the mild off-target effects observed for the rad27Δ and rad52Δ mutants in our in vivo assays. PTPD appears to be a species-specific hFEN1 inhibitor, but it may exhibit weak inhibition of other related yeast endonucleases that in turn causes negative genetic interactions in the rad27Δ and rad52Δ mutant strains. Accordingly, a humanized yeast system may be utilized to study the off-target effects of the HU-based compounds to filter those inhibitors that are most potent and specific to the desired target in an in vivo setting. The yeast cell-based assays presented in this study can be utilized to screen inhibitors of other cancer-relevant human targets such as the hERCC1/hERCC4 endonuclease complex (Elmenoufy et al. 2019), the ribonucleotide reductase hRRM2 (Zhou et al. 2013), the histone chaperone hASF1B (Seol et al. 2015), and the ubiquitin-conjugating enzyme hUBE2B (Sanders et al. 2013). Overall, these results highlight the utility of humanized yeast as a surrogate system and promote the necessity to complete testing of remaining yeast genes for complementation.
Acknowledgments

We thank Corey Nislow, Edward Marcotte, Aashiq H. Kachroo, and Tom Ellis for providing plasmids used in this study. This work was supported by grants to P.H. from the Canadian Institutes of Health Research (CIHR) (MOP 38096 and FDN 148449). A.H. is supported by doctoral awards from CIHR and The University of British Columbia.

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Communicating editor: Stanley Fields