A Functional Link Between Bir1 and the Saccharomyces cerevisiae Ctf19 Kinetochore Complex Revealed Through Quantitative Fitness Analysis

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ABSTRACT The chromosomal passenger complex (CPC) is a key regulator of eukaryotic cell division, consisting of the protein kinase Aurora B/Ipl1 in association with its activator (INCENP/Sli15) and two additional proteins (Survivin/Bir1 and Borealin/Nbl1). Here, we report a genome-wide genetic interaction screen in Saccharomyces cerevisiae using the bir1-17 mutant, identifying through quantitative fitness analysis deletion mutations that act as enhancers and suppressors. Gene knockouts affecting the Ctf19 kinetochore complex were identified as the strongest enhancers of bir1-17, while mutations affecting the large ribosomal subunit or the mRNA nonsense-mediated decay pathway caused strong phenotypic suppression. Thus, cells lacking a functional Ctf19 complex become highly dependent on Bir1 function and vice versa. The negative genetic interaction profiles of bir1-17 and the cohesin mutant mcd1-1 showed considerable overlap, underlining the strong functional connection between sister chromatid cohesion and chromosome biorientation. Loss of some Ctf19 components, such as Iml3 or Chl4, impacted differentially on bir1-17 compared with mutations affecting other CPC components: despite the synthetic lethality shown by either iml3Δ or chl4Δ in combination with bir1-17, neither gene knockout showed any genetic interaction with either ipl1-321 or sli15-3. Our data therefore imply a specific functional connection between the Ctf19 complex and Bir1 that is not shared with Ipl1.

KEYWORDS Bir1 Chromosome biorientation Kinetochore Iml3-Chl4 complex yeast

To maintain genomic integrity, it is essential that every chromosome be faithfully transmitted to both progeny during cell division. Genomic instability is a characteristic of cancer cells, and chromosome number alterations (aneuploidy) caused by gain or loss of chromosomes are thought to be one of the driving forces behind tumor progression (Hanahan and Weinberg 2011). To help ensure accurate chromosome segregation, sister chromatids generated by DNA replication are held together by protein complexes termed cohesin. The sister kinetochores, multiprotein complexes assembled at sister centromeres to mediate their attachment to microtubules (Lampert and Westermann 2011; Santaguida and Musacchio 2009), become linked to microtubules emanating from opposite spindle poles as they align on the mitotic spindle during metaphase (Nasmyth and Haering 2009). This state of attachment (chromosome biorientation) ensures that when cohesin is removed as cells enter anaphase, sister chromatids are pulled in opposite directions and each daughter receives exactly one copy of each chromosome (Tanaka et al. 2005).

Aurora B protein kinase has emerged over the past 15 yr as a key regulator promoting chromosome biorientation (Tanaka et al. 2005). Although there is an intrinsic bias favoring bioriented attachment of sister chromatids to the mitotic spindle that is most readily seen when the spindle pole bodies (SPBs) have already separated (Indjeian and Murray 2007; Verzijlbergen et al. 2014), achievement of biorientation is not automatic and attachment errors occur that would lead to chromosome mis-segregation if they were left uncorrected. Aurora B kinase corrects such errors, promoting detachment of incorrect attachments through phosphorylation of proteins at the kinetochore, such that correct attachments have a chance to replace them (Liu et al. 2009; Tanaka et al. 2002). Aurora B/ipl1 kinase forms part of the chromosomal passenger complex (CPC; see Ruchaud et al. 2007) together with three other conserved proteins (yeast names in parentheses): INCENP (Sli15), Survivin (Bir1), and Borealin (Nbl1). INCENP, Survivin, and Borealin associate via a triple helical interaction (Jeyaprakash et al...
2007) and INCENP contains a domain that binds to and activates Aurora B kinase (Kang et al. 2001). Error correction by Ipl1 kinase is an essential process and yeast cells show massive mis-segregation of chromosomes during division in its absence (Biggins et al. 1999). In addition to the CPC, efficient chromosome biorientation also requires the accumulation of cohesin around the centromere in yeast (pericentric cohesin), as well as pericentric cohesin and the protein Sgo1, which also interacts with this region of yeast chromosomes (Marston 2015). The contribution of pericentric cohesin, condensin, and Sgo1 may be to enforce a geometry that underlies the intrinsic bias toward chromosome biorientation (Verzijlbergen et al. 2014), while Sgo1 may be needed to sense when sister kinetochores are under tension from the mitotic spindle, thereby indicating that they are correctly bioriented (Marston 2015). The CPC-mediated error correction mechanism has generally been considered to involve inner centromere–localized Aurora B/Ipl1 (see Lampson and Cheeseman 2011). In yeast, CPC interaction with the inner centromere is targeted by Bir1 through its interactions with Ndc10 (Cho and Harrison 2012; Yoon and Carbon 1999) and with histone H2A phosphorylated on Ser-121 by Bub1 kinase (Kawashima et al. 2010). However, the importance of inner centromeric localization of the CPC has recently been called into question by the surprising finding that the Ipl1-Sli15 complex in yeast can still provide error correction, even in the absence of Bir1 or Nbl1, if it is delocalized from kinetochores by deletion of the first 228 residues of Sli15 that normally anchor Ipl1-Sli15 to Bir1 and Nbl1 (Campbell and Desai 2013; Fink et al. 2017; Jeyaprakash et al. 2007).

We previously generated a temperature-sensitive allele (birt1-17) supporting normal proliferation and chromosome biorientation at 26°C, but which fails to proliferate and shows a chromosome biorientation defect at 37°C (Makrantoni and Stark 2009). bir1-17 contains 11 point alterations within the C-terminus half of the protein, seven of which are localized within the C-terminus 297 residues of Bir1 that can provide its essential function (Widlund et al. 2006). Five point alterations are within the C-terminus 228 residues of Bir1 that interact strongly with both Nbl1 and Sli15 (Nakajima et al. 2009), and two lie within residues 889–941 that correspond to a domain proposed to form the triple helical interaction that is conserved in the human CPC (Jeyaprakash et al. 2007; Nakajima et al. 2009). One of these (L924S) affects a hydrophobic residue that is directly involved in the triple helical interaction, and is also mutated in two other conditional bir1 alleles (Shimogawa et al. 2009). Thus bir1-17 is likely to affect the interaction of the mutant protein with the other CPC components, although we have not examined this directly. Some of the point mutations are also located within a region of Bir1 that is known to interact with Ndc10 (Thomas and Kaplan 2007).

To understand better the role of Bir1 and the proteins and processes with which it interacts, we carried out a genome-wide synthetic interaction screen using the bir1-17 mutant. We found that the bir1-17 mutant is strongly enhanced by mutations affecting components of the Ctf19 kinetochore complex, including Chl4 and Iml3, and in the W303 background both chlΔ4 and imlΔ2 are synthetic lethal with bir1-17. Surprisingly, the synthetic lethal interactions between bir1-17 and either chlΔ4 or imlΔ2 are specific to bir1-17 and were not seen with either sli15-3 or ipl1-321, which each confer a much stronger Ts− phenotype than bir1-17 (Makrantoni and Stark 2009). The yeast Ctf19 complex is a group of inner kinetochore proteins that are analogous to the CCAN complex of metazoan kinetochores (Lampert and Westermann 2011; Santaguida and Musacchio 2009). Most yeast Ctf19 complex components are nonessential for proliferation, although gene knockouts confer elevated chromosome mis-segregation and reduced association of cohesin, condensin, Sgo1, and Ipl1 with centromere-proximal (pericentric) chromatin (Kiburz et al. 2005; Fernius and Marston 2009; Verzijlbergen et al. 2014). Our data therefore imply a specific functional connection between Bir1 and these two Ctf19 complex components that is critical for CPC function, and supports the notion that the bir1-17 mutation affects CPC function in a fundamentally different way to ipl1 mutations that simply reduce its ability to phosphorylate its targets. Our findings are consistent with the notion that delocalization of the CPC from kinetochores may make cells more dependent on mechanisms involving the Ctf19 complex that impart the intrinsic bias toward biorientation.

MATERIALS AND METHODS

Yeast strains and general methods

Basic yeast methods, growth media, and routine recombinant DNA methodology were performed as previously described (Amberg et al. 2005; Gietz et al. 1992). Unless stated otherwise, all yeast strains used in this study (Table 1) are derivatives of W303-1a (Thomas and Rothstein 1989) and have the following markers: ade2-1 his3-11, 112 trp1-1 ura3-1 can1-100 sa1-1 d2 Gal*. However, synthetic interactions screening was performed as previously described, using the BY strain background for reasons of strain compatibility with the genome-wide gene knockout collection (Addinall et al. 2011). To verify genetic interactions detected in the BY background, deletion strains were made W303 background by using the pFA6a-HIS3MX6 cassette as previously described (Longtine et al. 1998), and then crossed with bir1-17 in the same background. Deletion of IRC15 was performed such that the last eight codons of CTF19 (which overlap with IRC15) were retained.

Chromatin immunoprecipitation

Cohesin association with centromeric, pericentric, and arm sequences from chromosome IV was assessed in strains expressing HA-tagged Mcd1 using chromatin immunoprecipitation with anti-HA antibody (clone 12CA5) followed by qPCR analysis, performed as previously described (Fernius and Marston 2009; Fernius et al. 2013), using a Roche LightCycler and Express SYBR Green reagent (Invitrogen). PCR primers are listed in Supplemental Material Table S1.

Quantitative fitness analysis using bir1-17

To generate a bir1-17 strain in the S288C background suitable for synthetic gene array (SGA) screening, Y1082 was first transformed with a PCR fragment amplified from pFA6a-HpHMX6 (Hentges et al. 2005) using primers #536 and #537 (Table S1), such that the Hph marker

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replaced the region 236–297 bp upstream of the **BIR1** open reading frame (ORF), generating VMY165. This strain was next transformed with a PCR fragment made using primers #208 and #575 (Table S1) to amplify the **bir1-17**:NatMX construct from VMY26, in which the region 49–71 bases downstream of the **bir1-17** ORF was replaced by the NatMX marker from pAG25 (Goldstein and McCusker 1999). This
generated VMY179, from which the *bir1-17* region was amplified and sequenced to verify presence of all of the base changes in *bir1-17* (Makrantoni and Stark 2009). Figure S1 shows the doubly marked *bir1-17* locus generated. Flanking *bir1-17* with two different markers and then selecting for both during SGA greatly reduced the possibility that *bir1-17* could be separated from its markers by recombination. VMY179 grew normally at temperatures below 37°C but, while still clearly temperature-sensitive, showed some growth at 37°C, particularly when arrayed by pinning.

SGA analysis by crossing VMY179 to the systematic yeast gene deletion collection was performed as already described (Addinall et al. 2008, 2011). Quantitative fitness analysis (QFA) of the double mutants to generate strain fitnesses and genetic interaction strengths (GIS) was performed as previously reported (Addinall et al. 2011), by screening at 20, 27, and 37°C. Fitness and GIS calculations, including t-tests for the significance of GIS, were carried out and plots were generated using the QFA R package (version 0.0-43; http://qfa.r-forge.r-project.org/). Four replicate *bir1-17* strain (VMY179) crosses and eight replicate control strain DLY4242 (*ura3::NatMX*) crosses were analyzed. QFA data are summarized in Tables S2–S4 in File S2, following removal of all genes tightly linked to the query *bir1-17* mutation (i.e., located within 20 kb of *bir1-17* on chromosome X) and a standard set of genes related to the genetic selections used in SGA that are therefore incompatible with SGA (ARG82, ARG5, ARG4, ARG2, ARG3, ARG81, ARG80, ARG7, ARG1, ARG8, HIS7, HIS4, HIS2, HIS1, HIS6, HIS5, LEU2, LEU1, LEU5, LEU3, LEU4, LEU9, LYS2, LYS21, LYS20, LYS14, LYS4, LYS5, LYS12, LYS1, LYS9, and CCS1). To identify potentially significant phenotypic enhancers and suppressors, double mutants with p-value [false discovery rate (FDR) corrected p-value ≤ 0.05] and either a negative GIS (enhancers) or positive GIS (suppressors) were selected (Tables S5–S8 in File S2). GO terms enriched in the enhancer and suppressor gene subsets were determined using the GO Term Finder (version 0.83; Cherry et al. 2004), as implemented by the Saccharomyces Genome Database (SGD; Cherry et al. 2012, queried December 2016) with a p-value cut-off of ≤0.01. All recognized *Saccharomyces cerevisiae* nuclear-encoded ORFs within the systematic deletion collection, but lacking the *bir1-17*-linked genes and the SGA-incompatible genes listed above, were used as the background set for determining GO term enrichment (4235 genes; the full list is shown in each of Table S2, Table S3, and Table S4 in File S2). For further analysis of strong negative genetic interactors (GIS ≤ −25) identified at 20 or 27°C, growth of the individual control and *bir1-17* double mutants was examined. Where at least three out of four *bir1-17* *yfgΔ* replicates failed to grow but at least six out of eight control *ura3::NatMX* *yfgΔ* replicates grew, then the high negative GIS was considered to represent a synthetic lethal interaction (*yfgΔ*: your favorite gene deletion; used to indicate one of the ~4200 viable yeast gene deletions from the systematic deletion collection). Fitness plots were generated from the QFA data using iRVis (http://qfa.r-forge.r-project.org/vis Tool/), which is a part of the QFA software package, with significant negative and positive genetic interactors (i.e., q-values of ≤ 0.05 defined by t-test) colored blue and red, respectively.

### Data availability

Yeast strains are available on request. File S1 contains detailed descriptions of all supplemental files. File S2 contains Tables S2–S8. File S3 summarizes GO analysis of *bir1-17* enhancers and File S4 summarizes GO analysis of *bir1-17* suppressors. The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article and the supplemental material.

### RESULTS

**QFA identifies gene deletions that interact with *bir1-17***

To identify enhancers and suppressors of *bir1-17* that might indicate specific functional interactions, we used SGA technology to cross *bir1-17* to the collection of ~4200 viable systematic gene deletion strains, followed by QFA (Addinall et al. 2011) to identify suppressing or enhancing genetic interactions. *bir1-17* was originally isolated in the W303 genetic background and confers a recessive, temperature-sensitive growth defect that is clearly evident at 37°C (Makrantoni and Stark 2009). To investigate the best approach for identifying *bir1-17* suppressors and enhancers, we first compared growth of the S288C background control and *bir1-17* strains after arraying by either pinning or spotting. Figure S2A shows that when strains were arrayed by pinning, the fitness defect of *bir1-17* was hard to detect even at higher incubation temperatures (Figure S2A). In contrast, when dilutions of the two strains were arrayed by spotting onto the screening plates then the fitness defect of *bir1-17* was readily detectable at 37°C (Figure S2B). We therefore carried out QFA analysis by spotting rather than pinning the arrays of control and *bir1-17* double mutants generated by SGA.

Double *bir1-17* *yfgΔ* mutants were generated at 23°C. QFA was subsequently performed by spotting out and screening growth at 20, 27, and 37°C, calculating the GIS and q-value (FDR-corrected p-value) for each double mutant to indicate the magnitude of the genetic interaction between *bir1-17* and each *yfgΔ* gene knockout and its statistical significance, respectively (Tables S2–S4 in File S2; data ranked in ascending order of GIS, starting with the most negative interactions). Since *bir1-17* cells spotted at 37°C show a clear growth defect, we chose to focus primarily on this dataset. A q-value threshold of ≤0.05 was applied to identify a subset of statistically significant genetic interactions at each screening temperature. QFA data were summarized in the form of fitness plots, in which the fitness of each *bir1-17* *yfgΔ* strain is plotted against the fitness of the corresponding control *ura3::yfgΔ* strain.

Figure 1 shows the fitness plot for *bir1-17* *yfgΔ* mutants screened at 37°C, indicating all statistically significant enhancers (negative GIS; blue triangles) and suppressors (positive GIS; red triangles). Table S5 and Table S8 in File S2 list the statistically significant *bir1-17* enhancers and suppressors, respectively, identified by screening at 37°C, while Table S6 and Table S7 in File S2 present the statistically significant enhancers at 20 and 27°C for comparison. In the fitness plot, the dashed gray line indicates where points should lie when deletion mutants show identical fitness in combination with either *bir1-17* or the control *ura3Δ* mutation (the line of equal growth). The regression line of the actual data points is indicated by the solid line. Downward displacement of this regression line away from the line of equal growth (Figure 1) is consistent with the temperature-sensitive phenotype of *bir1-17* that was clearly evident following spotting out under QFA conditions (Figure S2), and is in contrast to the fitness plots from the 20 and 27°C screens (Figure S3).

To look in an unbiased manner for relationships between genes identified as statistically significant enhancers and suppressors of *bir1-17* at 37°C, we searched for GO terms within the process, function, and component ontologies that showed significant enrichment in the enhancers and suppressors. We focused primarily on strong interactions ([GIS] ≥ 25; mapped onto the 37°C fitness plot in Figure S4), but also searched using enhancers and suppressors with [GIS] ≥ 10 for comparison. These data are summarized in File S3 (enhancers) and File S4 (suppressors). We also manually examined the position on the 37°C fitness plot of the members of each of the core protein complexes defined by Benschop et al. (2010), as a means of identifying consistent patterns of genetic interaction with *bir1-17* that might reflect functional
significant enhancers at all. It is striking that in contrast to the two lower temperature screens, the 37°C screen placed knockouts of genes encoding all the Ctf19 complex components except Nkp1 and Nkp2 in a tight cluster at the lower right of the plot, indicating that the knockouts had little or no defect in the control (ura3Δ) background, but in contrast had a very strong defect when combined with bir1-17 (see Figure S3). Thus, screening at 37°C when double mutants are close to the bir1-17 maximum permissive temperature greatly assisted in identifying genetic interactions between the core components of the Ctf19 complex and bir1-17.

GO analysis of the strong bir1-17 enhancers (GIS ≤ −25) identified at 37°C (see Table S5 in File S2) highlighted a number of specific GO terms concerned with chromosome segregation, sister chromatid cohesion, and microtubule-based processes, as well as more general terms including nuclear division and chromosome organization that encompassed many of the strongest negative genetic interactions (File S3). In addition to the Ctf19 complex, this analysis highlighted genes encoding all the S. cerevisiae kinesin-like proteins except KIP1 (i.e., KIP2, KIP3, KAR3, and CIN8), VIK1 and CIK1 (encoding Kar3-associated proteins; Manning et al. 1999), BIK1 (a plus-end tracking protein related to CLIP-170; Berlin et al. 1990; Blake-Hodek et al. 2010), TUB3 (α-tubulin), CIN1 (encoding a β-tubulin folding factor; Hoyt et al. 1990, 1997) and KAR9 (encoding a spindle positioning factor; Beach et al. 2000; Miller et al. 2000), although kar9Δ was inviable at 20 and 37°C but was synthetic lethal with bir1-17 at 27°C. bim1Δ, removing another plus-end tracking protein that binds to Kar9 and is also involved in spindle positioning (Beach et al. 2000; Miller et al. 2000), was also clearly a strong bir1-17 enhancer at the two lower temperatures, although it fell outside our statistical significance cut-off at 37°C. Thus cells lacking proper Bir1 function appear to become particularly dependent on the normal functioning of kinesins and microtubules.

GO analysis, together with consideration of the core protein complexes defined by Benschop et al. (2010), also identified knockouts of genes encoding any of the three members of the Ctb8/Ctf18/Dcc1 complex that is related to replication factor C (RFC3Δ) as enhancers of bir1-17 (although ctf18Δ fell just outside our cut-offs of q ≤ 0.05 and GIS ≤ −25 for significant strong enhancers). All three members of the Tof1/Mrc1/Csm3 complex that acts at stalled replication forks to promote sister chromatid cohesion (Bando et al. 2009; Tourriere et al. 2005; Xu et al. 2004) were also highlighted (although tof1Δ fell just outside our q-value cut-off). Like the Tof1 complex, the Ctf8 complex is also involved in sister chromatid cohesion (Mayer et al. 2001, 2004), highlighting the link between this process and CPC-dependent error correction. Examination of the core protein complexes also revealed negative enhancement of bir1-17 by those viable knockouts in the systematic collection that affected RFC6Δ (RAD24), RFC8Δ (ELG1), a PCNA-like clamp (RAD17 and possibly DDC1) and DNA polymerase epsilon [DPB3, YBR277c (ΔDPB3), and DPB4 if the q-value cut-off is relaxed].

Broadening the GO analysis to consider enhancers with a GIS ≤ −10 did not change this overall picture but gave greater emphasis to some categories such as genes involved in chromatin modification (components of the Set3C, ISW, Compass, Ada, and Rpd3S complexes), histone exchange (SWC5, VPS71, and VPS72), tRNA wobble uridine modification (components of Elongator, ATS1, KTT12, URM1, UBA4, NCS2, NCS6, and SAP190), iron transport (FET3 and FTR1), and peroxisomal function. Multiple components in each of these categories were strong, significant enhancers of bir1-17 at 37°C (Table S5 in File S2). While a potential functional connection between Bir1 and either iron transport or peroxisomes is not at all obvious, many of these other enhancers may function indirectly by interactions between Bir1 and specific cellular processes. Table 2 summarizes the proportion of bir1-17 enhancers or suppressors identified at each screening temperature. Around 3% of yfgΔ knockouts strongly enhanced bir1-17 in the 37°C screen (GIS ≤ −25), while 2.2% of knockouts strongly suppressed (GIS ≥ 25). This was in contrast to the 20 and 27°C screens, where the proportion of strong interactions was far less.

**Phenotypic enhancers of bir1-17**

It was quickly apparent from looking at the bir1-17 enhancers identified at 37°C (Table S5 in File S2) that knockouts of almost any of the non-essential components of the Ctf19 kinetochore complex (CHL2, CTF3, CTF19, IML3, IRC15, MCM16, MCM21, MCM22, and NKP1; see Biggins 2013) strongly enhanced bir1-17; only Nkp2 was not identified as an enhancer. IRC15 is included here because of the overlap of its full-length deletion with CTF19, as discussed below. All of these knockouts except nkp1Δ fell into the strong enhancer category (i.e., GIS ≤ −25), and five out of the six strongest enhancers were members of the Ctf19 complex (Table S5 in File S2). Only MCM21 and CTF19, encoding the two non-essential members of the COMA subcomplex (Am8 and Okp1 are both essential; De Wulf et al. 2003; Ortiz et al. 1999), were among the strongest enhancer of bir1-17 at all three screening temperatures. The other members of the Ctf19 complex were either weaker enhancers at 20°C and 27°C than at 37°C, or in some cases, not identified as...
altering the pattern of expression of proteins that have a direct functional relationship with Bir1. Other notable strong enhancers not falling into any of the above-mentioned categories included knockouts of the CENP-T-related kinetochore component \( \text{CNN1} \) that interacts with the \( \text{Ndc80} \) kinetochore subcomplex (Bock et al. 2012; Malvezzi et al. 2013) and the \( \text{CPR6} \) peptidyl-prolyl cis-trans isomerase and its interacting protein encoded by \( \text{STI1} \) (Mayr et al. 2000). Figure S6 shows these groups of genes mapped onto the 37\(^\circ\)C fitness plot as an indicator of how consistently they interacted with \( \text{bir1-17} \) in the screen.

### Phenotypic suppressors of \( \text{bir1-17} \)

QFA carried out at 37\(^\circ\)C identified many potential suppressors of \( \text{bir1-17} \) that caused relief of the temperature-sensitive growth defect, including 94 strong suppressors (GIS \( \geq 25 \); Table S8 in File S2). GO analysis of these genes (File S4) revealed highly significant enrichment for genes encoding components of the large ribosomal subunit (LSU) or proteins involved in its rRNA processing and assembly, as well as for genes involved in mRNA catabolism, and in particular nonsense-mediated mRNA decay (NMD). This was further supported by our analysis of the core protein complexes of Benshop et al. (2010). Thus four out of the six strongest suppressors and almost one-third of all the strong (GIS \( \geq 25 \)) suppressors could be assigned roles either in ribosome biogenesis or as components of the ribosome. Strikingly, the vast majority of knockouts affecting the ribosome were specific for the LSU; we isolated 27 \( \text{RPL} \) gene deletions (removing LSU proteins) but only three \( \text{RPS} \) gene deletions (removing small ribosomal subunit proteins) as statistically significant \( \text{bir1-17} \) strong suppressors. Taking the unbiased approach of mapping all genes annotated as \( \text{RPL} \) and \( \text{RPS} \) in SGD onto the 37\(^\circ\)C fitness plot, we confirmed that genes in these two groups in general behaved very differently when knocked out and combined with \( \text{bir1-17} \) (Figure S7, compare A and B), while Figure S7C shows that the vast majority of all gene knockouts affecting either biogenesis of the LSU or LSU components caused phenotypic suppression of \( \text{bir1-17} \).

Knockouts of the NMD genes \( \text{EBS1, NMD2, NAM7, and UFP3} \) were all within the strong suppressor set (\( \text{nam7} \Delta \) was the second strongest suppressor), while the remaining components of the NMD pathway present in the systematic deletion collection (\( \text{DCN1 and NMD4} \)) were also statistically significant suppressors but falling just below our GIS \( \geq 25.0 \) cut-off (Table S8 in File S2). \( \text{SK12 and SK13} \), encoding two components of the Ski complex that are involved in a variety of RNA decay processes, including NMD and processes mediated by the exosome, were also identified. The remaining functionally related genes (\( \text{RRP6, SK17, SK18, and YKL023w} \)) were also suppressors falling slightly below our GIS cut-off. Figure S8A summarizes how the SK1, NMD, and exosome gene knockouts mapped onto the 37\(^\circ\)C fitness plot.

Although GO terms directly related to cell division were not specifically highlighted by our GO analysis even when suppressors with GIS \( \geq 10 \) were included, one gene encoding a kinetochore component (\( \text{YBP2} \)) was found within the top 30 suppressors (File S4). Mapping the core protein complexes defined by Benshop et al. (2010) onto the 37\(^\circ\)C fitness plot also identified mutations affecting the COP9 signalosome as suppressors of \( \text{bir1-17} \). The COP9 signalosome removes the NEDD8 homolog **Rub1** from the yeast cullin **Cdc53** and is required for cell cycle regulation at the G1/S boundary (Wee et al. 2002). Thus, both **csi1Δ** and **pci8Δ** were strong significant suppressors, while **csp9Δ, rti1Δ, and rti2Δ** were suppressors that fell just outside our GIS and/or q-value cut-offs (Figure S8B).

The basis for suppression by each of these classes of genes is not yet clear, but suppression by the first two groups of gene knockout is most likely related to alterations in the expression of proteins caused by changes in mRNA stability or alterations in the ribosome population. Since \( \text{bir1-17} \) is a temperature-sensitive loss of function mutant, it may be that these knockouts lead to elevated \( \text{bir1-17} \) protein levels at higher temperatures that can compensate for the effect of the mutations it contains. In a previous QFA analysis, both NMD gene deletions and \( \text{RPL} \) (but not \( \text{RPS} \)) gene deletions were also found to suppress the temperature-sensitivity of a **cdc13-1** query mutation (Addinall et al. 2011). In this case, the NMD deletions were shown to operate through affecting levels of another protein (**Stn1**), which like **Cdc13**, is specifically involved in telomere function (Addinall et al. 2011). Conversely, both NMD and **RPL** gene deletions enhanced the phenotype of **yku70Δ** in a parallel QFA analysis (Addinall et al. 2011). It is therefore possible that loss of either the NMD pathway or specific **RPL** genes may lead to generalized effects on gene expression that can suppress or enhance specific mutations through effects on expression of functionally related genes.

### The genetic enhancement profiles of \( \text{bir1-17} \) and \( \text{ipl1-321} \)

**ipl1-321** shows both common and distinct features, many of which are shared with **mcd1-1**

Since **ipl1** functions together with **Sli15**, **Bir1**, and **Nbl1** within the yeast CPC complex, it might be expected that strains with loss-of-function mutations in each of these genes would show synthetic lethality or strong negative genetic interactions with a common set of nonessential gene knockouts that reflect their shared functional roles. Comprehensive analysis has yet to be performed with conditional alleles of either **sli15Δ** or **nbl1Δ**, but our study now enables comparison of the strong genetic enhancers of both **ipl1-321** and **bir1-17** to be made. Figure 2 shows that 13 of the strong genetic interactions of **bir1-17** established in our work are indeed shared with the known synthetic lethal interactions of **ipl1-321** (colored yellow and red). Overlap between strong negative genetic interactors of **ipl1-321** and those of the cohesin mutant **mcd1-1** (also called **scc1-73**) was noted previously (Ng et al. 2009) and is also the case here for **bir1-17**, with nine strong negative genetic interactions shared by all three mutant alleles (colored red in Figure 2). These data underline the strong functional links between sister chromatid cohesion, chromosome biorientation, and faithful sister chromatid segregation that operate during cell division.

Remarkably, the **ipl1-321** and **bir1-17** alleles each individually show at least as many strong negative genetic interactions with **mcd1-1** that they do not share with each other. For example, strains lacking several nonessential components of the **Ctf19** complex that were identified in this study as the strongest negative enhancers of **bir1-17** are also

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**Table 2 Summary of \( \text{bir1-17} \) suppressors and enhancers**

<table>
<thead>
<tr>
<th>QFA Screening Temperature</th>
<th>Enhancers (%) ( \geq 25 )</th>
<th>Suppressors (%) ( \geq 25 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>GIS &lt; −10</td>
</tr>
<tr>
<td>37(^\circ)C</td>
<td>11.1</td>
<td>8.5</td>
</tr>
<tr>
<td>27(^\circ)C</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>20(^\circ)C</td>
<td>1.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\( ^a \)Based on SGA using 4235 gene deletions and requiring a q-value of < 0.05.
Figure 2 Comparison of the strong negative genetic interactors of ipi1-321, bir1-17, and mcd1-1 revealed by SGA analysis. Genes showing synthetic lethality or strong negative genetic interaction with two or more of ipi1-321, bir1-17, or mcd1-1 are connected to the relevant query mutations by lines that are color-coded according to the number of shared interactions as follows: yellow, negative genetic interactors shared by ipi1-321 and bir1-17; dark blue, negative genetic interactors shared by ipi1-321 and mcd1-1; light blue, negative genetic interactors shared by bir1-17 and mcd1-1; and red, negative genetic interactors shared by all three query genes. The gray box with dashed outline encloses genes encoding components of the Ctf19 complex. Strong negative genetic interactors shown in the diagram were defined as follows: ipi1-321, all genes identified by Ng et al. (2009) as ipi1-321 negative genetic interactors together with additional genes listed in SGD (Cherry et al. 2012, queried December 2016); mcd1-1, all genes identified by Ng et al. (2009) or present in the DRYGIN database (Koh et al. 2010, queried December 2016) as mcd1-1 negative genetic interactors, together with additional genes listed in SGD (Cherry et al. 2012, queried December 2016); bir1-17, all strong negative enhancers identified in this work at any of the three screening temperatures that were shared with at least one of the other two mutations (ipi1-321 or mcd1-1).
could be verified in W303 as *bir1-17* enhancers (*ski3Δ*, *vps71Δ*, and *yku70Δ*) but several others affecting the COMPASS (*bre2Δ*, *spt15Δ*), ISW (*chl4Δ*) and Rpd3S (*sin3Δ*) complexes showed little or no enhancement of *bir1-17* in W303.

Although we reproduced the negative genetic interaction between *bir1-17* and *irc15Δ* in W303 using a precise ORF knockout identical to that in the systematic collection used for the QFA screen (data not shown), this knockout also deletes the last eight sense codons of *CTF19*, with which *IRC15* overlaps on the opposite strand. Using a construct that removed the first 332 codons of the 499-codon *IRC15* ORF and thus left the *CTF19* ORF intact, we found no genetic interaction with *bir1-17* (Figure 3A), and so we conclude that *irc15Δ* was identified in our QFA screen because the systematic knockout interferes with Ctf19 function. It is also therefore likely that other mitotic phenotypes reported for *irc15Δ* mutants may result from effects on the overlapping *CTF19* gene rather than indicating functional consequences of *Irc15* loss.

**bir1-17, but not *ipl1-321*, is dependent on the *Iml3-Chl4* subcomplex for viability**

Given that we identified several verifiable strong negative genetic interactions in our QFA analysis between *bir1-17* and Ctf19 complex gene knockouts that were not seen in a similar *ipl1-321* screen (Ng et al. 2009), we looked in more detail at *iml3Δ* and *chl4Δ*. When *iml3Δ* and *chl4Δ* strains were crossed with *ipl1-321*, *ipl1-2*, and *sli15-3* mutants (all in the W303 background), viable double mutants were readily isolated in each case and grew essentially normally at 26°C, which is permissive for all three *Ts*− alleles (Figure 3B). Thus *bir1-17* strains show complete dependence on functional Iml3 and Chl4 in W303, whereas *ipl1-321*, *ipl1-2*, and *sli15-3* strains are virtually independent of Iml3 and Chl4 for normal proliferation. This confirms that at least some of the differences between the genetic interaction profiles of *ipl1-321* and *bir1-17* are genuine and implies that there is a fundamental difference in the way that the *ipl1* and *sli15* mutations affect CPC function in comparison with *bir1-17*.

The *sli15*Δ (**ANT**) allele removes the first 228 residues of Sli15 and thereby prevents its interaction with Bir1. Surprisingly, this is not lethal as anticipated, despite the delocalization of *ipl1* from kinetochores as a result of the truncation (Campbell and Desai 2013), leading to the idea that while targeting of the CPC to the kinetochore may be important for efficient chromosome biorientation, it is not essential for it to occur. However, the combination of *sli15*Δ (**ANT**) with either *ctf19Δ* or *mcm21Δ* was almost lethal (Campbell and Desai 2013). We therefore crossed *sli15*Δ (**ANT**) with *iml3Δ* and *chl4Δ* and, although the double mutants could be obtained, in contrast to *sli15-3* (Figure 3B), there was a clear negative genetic interaction in both cases and the *chl4Δ sli15*Δ (**ANT**) double mutant was inviable at 35°C, a temperature at which each single mutant grew normally (Figure 3C). Thus *bir1-17* and *sli15*Δ (**ANT**) share a clear negative genetic interaction with loss of either Iml3 or Chl4 that is not seen in *ipl1* mutants or *sli15-3*. Double *nbl1-6* *chl4Δ* and *nbl1-6* *iml3Δ* mutants could also be obtained, but again showed a strong negative genetic interaction at 35°C or above (Figure 3C). In summary, mutations that affect the targeting of the CPC [on *nbl1-6*, *sli15*Δ (**ANT**) and *bir1-17*] show strong negative genetic interactions with loss of either *IML3* or *CHL4*, whereas mutations primarily affecting CPC’s protein kinase activity (*ipl1-2*, *ipl1-321*, and *sli15-3*) do not.

**bir1-17 does not reduce accumulation of pericentromeric cohesion**

The Ctf19 kinetochore complex is important for establishment of pericentromeric cohesion, while Csm3 is needed for ensuring that
pericentromeric cohesin functions to hold sister centromeres together and shows an additive phenotype with mutations affecting the Ctf19 complex (Fernius and Marston 2009). We therefore examined whether the bir1-17 mutant might also have a defect in the accumulation of pericentromeric cohesin that could explain its strong negative genetic interactions with knockouts affecting the Ctf19 and Csm3-Mrc1-Tof1 complexes. After synchronizing cells in G1 and then releasing them at 37°C in the presence of benomyl and nocodazole, association of cohesin at the centromere, pericentromere, and arm regions of chromosome IV was quantified in metaphase-arrested cells by chromatin immunoprecipitation using HA-tagged Mcd1. Relative to a wild-type control, the bir1-17 strain showed no obvious defect in the association of cohesin with the centromeric and pericentromeric regions (Figure 4). In contrast, a chl4Δ strain showed a clear deficiency in the level of cohesin at the centromere and pericentromere but not in accumulation of cohesin on the chromosome arm, as found previously (Fernius and Marston 2009). Thus defective accumulation of cohesin around the centromere in bir1-17 is unlikely to provide an explanation for its strong negative interaction with other mutations affecting pericentromeric cohesion. However, we cannot exclude the possibility that, as in csm3 mutants (Fernius and Marston 2009), the pericentromeric cohesin that accumulates is not fully functional.

Sgo1 overexpression does not relieve the requirement for Chl4 and Iml3 in bir1-17 strains

Since Iml3 and Chl4 are also needed for pericentromeric accumulation of Sgo1, which is involved both in CPC recruitment and the bias of sister kinetochores to form bioriented attachments (Verzijlbergen et al. 2014), we tested whether boosting Sgo1 expression could suppress the synthetic lethality between iml3Δ or chl4Δ and bir1-17. To do this and to provide a platform for further analysis of the defect in the double mutants, we utilized the “anchor away” system, in which proteins that function within the nucleus can be excluded by the addition of rapamycin, thereby triggering conditional loss of function (Haruki et al. 2008). This was carried out in the context of a TOR1-1 background so that cells were resistant to growth inhibition by rapamycin, and rapamycin-induced effects can therefore be ascribed solely to nuclear exclusion of the protein of interest. To verify the use of this approach with the Ctf19 kinetochore complex, we tagged each of the two essential Ctf19 complex members (Ame1 and Okp1) with FRB-GFP and demonstrated that cells could no longer grow when rapamycin was added, while strains in which the nonessential Iml3 and Chl4 were FRB-tagged allowed robust growth on rapamycin (Figure S10A). This analysis also confirmed that nuclear exclusion of Iml3 and Chl4 did not interfere with other essential components of the kinetochore, for example through them “piggy-backing” out of the nucleus with the tagged protein. Microscopy of both the Iml3-FRB-GFP and Chl4-FRB-GFP
strains confirmed that kinetochore localization of either tagged protein was lost within 50 min of rapamycin treatment (Figure S10B).

To test whether boosting Sgo1 levels could reverse the synthetic lethality between bir1-17 and loss of Iml3 or Chl4, bir1-17 was introduced into the iml3-FRB-GFP and Chl4-FRB-GFP strains, and then a galactose-inducible GAL-SGO1 construct introduced. As shown in Figure 5, addition of rapamycin to the growth medium recapitulated the synthetic lethal phenotype of bir1-17 with iml3Δ or chl4Δ gene knockouts. The rapamycin-induced lethality was not overcome by induction of the SGO1 overexpression construct. Furthermore, inducing SGO1 expression appeared, if anything, somewhat detrimental to the growth of strains lacking nuclear Iml3 or Chl4. Iml3 and Chl4 both have a specific role in ensuring correct sister chromatid separation in meiosis II that is not necessarily shared with other Ctf19 components (Marston et al. 2004), but Figure 5 confirms that the synthetic lethality seen in genetic crosses (Table S9) is also seen in mitotically proliferating cells.

**DISCUSSION**

Here, we present the results of a QFA screen using the temperature-sensitive bir1-17 mutant that is defective in chromosome biorientation to identify both enhancers and suppressors of bir1-17. By screening close to the maximum permissive temperature for bir1-17 at 37°C, many strong enhancers and suppressors of bir1-17 were identified. Furthermore, QFA performed following spotting, was easily able to identify fitness differences between strains that pinning (SGA) could not (see Figure S2). The strong enhancer set contained groups of genes involved in chromosome segregation, sister chromatid cohesion, and microtubule-based processes, consistent with the known function of Bir1 within the CPC, along with additional categories. Our QFA analysis was therefore successful in identifying strong genetic interactions with a subset of genes that, like BIR1, are involved in processes related to chromosome segregation. In contrast, only a very small number of enhancers were identified that affected fitness at 20 and/or 27°C, temperatures that are fully permissive for bir1-17, and overall only four true synthetic lethal interactions were observed (with kar3Δ, coq2Δ, sac1Δ, and yme1Δ) within the statistically significant strong enhancers that we identified. With the exception of kar3Δ (see above), none of these genes show any obvious functional connection with BIR1.

The negative genetic interaction profiles of ipl1-321, bir1-17, and mcd1-1 showed considerable overlap, consistent with the importance of sister chromatid cohesion for chromosome biorientation, and the bir1-17 screen highlighted the importance of all the core components of the Ctf19 kinetochore complex in cells where Bir1 function is compromised. All of the interactions between bir1-17 and Ctf19 complex members and several of the other interactions identified by QFA involving the kinesin Cin8, the Tof1 complex, RFC\textsubscript{cmtt}, and tRNA wobble uridine modification were independently verified in the W303 background. Despite the expected overlap that we saw between the enhancers of ipl1-321 and bir1-17, given that Ipl1 and Bir1 function together within the CPC, we nonetheless identified several genes, of which IML3 and CHL4 are notable examples, that become essential only in bir1-17 and not in ipl1-321 and thus support the notion that the ipl1 and bir1 mutations affect CPC function in somewhat different ways.

Unlike the enhancers, GO analysis of the bir1-17 suppressors identified by QFA did not highlight chromosome or microtubule-based functions, but instead identified genes involved in mRNA catabolism (principally the NMD pathway) or genes encoding LRSU proteins as bir1-17 suppressors. While we cannot exclude the possibility that these suppressors impinge directly on kinetochore function, the CPC or sister chromatid cohesion, we consider it more likely that they relieve the temperature-sensitivity of bir1-17 by altering the expression level of one or more proteins that are relevant to Bir1 function, and that these suppressors therefore act in a less direct manner. Loss of the NMD pathway and components of the LRSU have been isolated as suppressors in other unrelated SGA screens, emphasizing their potential to act.
pleiotropically (Addinall et al. 2011). These components may therefore play a role in restricting the viability of partial loss of function mutations in a wide range of essential genes, countering the mutational buffering capacity provided by heat shock proteins that allows such mutations to survive (Rutherford 2003).

A single kinetochore protein gene knockout (ybp2Δ) was identified as a suppressor of bir1-17. Ybp2 shows interactions with the COMA and Ndc80 kinetochore subcomplexes and in its absence there are changes in the interactions between members of the KNL [Kn1 (Spc105)-Ndc80-Mis12] network in the kinetochore (Ohkuni et al. 2008). This is reminiscent of cnn1Δ, which we identified as a strong enhancer of bir1-17: Cnn1 also interacts with the Ndc80 complex and cnn1Δ also affects interactions within the KNM network within the kinetochore (Bock et al. 2012). Identification of Cnn1 and YBP2 as strong genetic interactors of BIR1 might therefore indicate a role of the CPC in modulating interactions within the KNM network and is consistent with the notion proposed by Bock et al. (2012) that Cnn1 and Ybp2 could act in overlapping pathways that regulate KNM interactions during the cell cycle.

Although the Ctf19 complex is a component of the kinetochore, it is now clear that it plays a key role in providing a signal for the deposition of pericentromeric cohesin on chromosomes (Fernius and Marston 2009), which in turn leads to the recruitment of Sgo1 and condensin (Verzijlbergen et al. 2014). The pericentromeric region has a specialized structure (Yeh et al. 2008) and it is likely that the accumulation of cohesin and condensin in this region is part of a mechanism that provides an intrinsic bias toward bioriented attachment of sister chromatids to spindle microtubules. In3 and Chl4 form a heterodimer (Hinshaw and Harrison 2013) that is a peripheral component of the Ctf19 complex, based on the assembly dependencies that have been established for Ctf19 complex (Pot et al. 2003). However, loss of Im3 or Chl4 is sufficient to disrupt the association of cohesin, condensin, and Sgo1 with the pericentromere, despite not affecting the interaction of other Ctf19 complex members with the kinetochore. Why then, should loss of Im3 and Chl4 only be a problem in combination with bir1-17 and not ipil-321? In ipil-321, the mutation affects the catalytic subunit of the CPC (Biggins et al. 1999) but it can most likely still be targeted to the kinetochore through its interactions with Sli15, Nbl1, and Bir1. While the bir1-17 mutation does reduce Ipl1-dependent kinase activity, it also causes significant delocalization of Ipl1 from the kinetochore (Makrantoni and Stark 2009), and under circumstances where Ipl1 is delocalized, we now know that the Ctf19 complex becomes essential for viability (Campbell and Desai 2013). Thus, although kinase function is reduced in ipil-321, because it can be targeted to the kinetochore it may be sufficient to overcome loss of any intrinsic bias toward biorientation that requires Im3 and Chl4-dependent accumulation of cohesin, condensin, and Sgo1 at the pericentromere. Conversely, in bir1-17 strains as in nb1-6 strains (Nakajima et al. 2009) and sli15ΔNT strains (Campbell and Desai 2013), delocalization of the CPC from kinetochores may make CPC-dependent error correction less efficient, and cells may now rely much more on the intrinsic bias toward biorientation that ultimately relies on Ctf19 complex-dependent events at the pericentromere. Interestingly, the intrinsic bias toward biorienting chromosomes is greater, and hence the need for CPC-mediated error correction much lower, when microtubule attachment occurs after the SPBs have separated (Indjeian and Murray 2007). This feature may explain the negative genetic interactions between bir1-17 and either kar3Δ or cin8Δ. These two genes encode motor proteins that are involved in spindle pole separation and both knockouts lead to short spindles (Gardner et al. 2008; Hoyt et al. 1992; Roof et al. 1992; Saunders and Hoyt 1992), which may reduce the intrinsic biorientation bias and lead to a much greater requirement for CPC-mediated error correction.

Since ctf19Δ mutant kinetochores lack both Iml3 and Chl4 (Pot et al. 2003), the synthetic lethality between ctf19Δ and bir1-17 could, in principle, be explained solely on the basis of loss of Chl4 and Im3, and this could also be the case for some or all of the other deletions of Ctf19 components that share this phenotype. Why then, might loss of some core Ctf19 complex components such as MCM21 also lead to inviability in the ipil-321 mutant? Perhaps absence of components such as MCM21 leads to a significantly greater loss of the intrinsic bias toward bi-orientation, or alternatively, these inner components of the Ctf19 complex may have additional roles in kinetochore function, as proposed for their higher eukaryotic counterparts (Suzuki et al. 2014), which are separate from their requirement to signal cohesin and condensin deposition at the pericentromere and that lead to reduced kinetochore function when they are absent.

Although we can account for the known phenotypes of the bir1-17 mutation based on Bir1 being a component of the yeast CPC (Makrantoni and Stark 2009), it is possible that some of the genetic interactions we have found might reflect losses of Bir1 that are independent of it being part of the canonical CPC (i.e., the ipil-321-Nbl1-Bir1 complex). It has been reported that a significant fraction of Bir1 is present in a complex with Sli15 (and possibly also Nb11) that do not contain Ipl1 (Sandall et al. 2006; Thomas and Kaplan 2007) and this complex has been implicated both in septic dynamics (Gillis et al. 2005; Thomas and Kaplan 2007) and as a tension sensor at the kinetochore (Sandall et al. 2006). We could not find any genes annotated in SGD as being involved in septic function among the bir1-17 enhancers, although five such genes (DMA1, ELM1, GIC2, RGA1, and SPR3) were identified as weak bir1-17 suppressors (Table S8 in File S2) and may relate to the ipil1-independent role in septic behavior proposed for Bir1 (Thomas and Kaplan 2007). If Sli15-Bir1 does constitute some form of tension-sensing linkage as proposed by Sandall et al. (2006), then it is also possible that the interactions we find with the Ctf19 complex could, in part, reflect a requirement for pericentromeric cohesion in promoting tension-sensing.

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