Degradation of Centromeric Histone H3 Variant Cse4 Requires the Fpr3 Peptidyl-prolyl *Cis–Trans* Isomerase

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ABSTRACT The centromeric histone H3 variant Cse4 in *Saccharomyces cerevisiae* is polyubiquitylated and degraded in a proteasome-dependent manner. We report here that the proline isomerase Fpr3 regulates Cse4 proteolysis. Structural change in Cse4 by Fpr3 might be important for the interaction between Cse4 and the E3 ubiquitin ligase Psh1.

ACCURATE chromosome segregation during mitosis and meiosis is a critical event in the transfer of genetic information to daughter cells. Loss or gain of chromosome is associated with cancer development and genetic disease (Yuen *et al.* 2005; Holland and Cleveland 2009). The centromere–kinetochore complex is required for faithful segregation. We previously used an *in vitro* kinetochore assembly system and identified the FK506 binding protein Fpr3 as a novel protein that associates with *CEN* DNA (Ohkuni and Kitagawa 2011). Fpr3 was also isolated by affinity purification of the Dsn1–Flag-tagged central kinetochore protein (Akiyoshi *et al.* 2010). These data strongly suggested that Fpr3 has a role in centromere and/or kinetochore function. In this study, we investigated the mitotic function of Fpr3.

FPR3 and *FPR4* encode two related prolyl isomerases that share 46% identity (Arevalo-Rodriguez *et al.* 2004). Fpr4 regulates histone H3 lysine 36 methylation and gene activity by its proline isomerase activity (Nelson *et al.* 2006). To characterize the function of Fpr3 and Fpr4, we first generated null mutants of the nonessential *FPR3* and *FPR4* genes, respectively (Supporting Information, Table S1) (Dolinski *et al.* 1997; Arevalo-Rodriguez *et al.* 2004). Single deletion strains (*fpr3*Δ and *fpr4*Δ) and double deletion strains (*fpr3*Δ *fpr4*Δ) did not show obvious growth phenotypes such as temperature or benomyl sensitivities (Figure S1). We also examined chromosome stability in *fpr3*Δ or *fpr4*Δ strains by a colony color assay (Figure 1A). Both *fpr3*Δ and *fpr4*Δ single deletion strains showed a moderate chromosome missegregation phenotype (chromosome fragment loss: 0.2% in *fpr3*Δ, 0.15% in *fpr4*Δ). Because Fpr3 is associated with centromeres (Akiyoshi *et al.* 2010; Ohkuni and Kitagawa 2011) and because Fpr3 and Fpr4 directly interact with histone H3 (Nelson *et al.* 2006), we tested a possibility that Fpr3 and/or Fpr4 regulate a centromeric histone H3 variant Cse4. Interestingly, we found that the endogenous protein level of Cse4 was increased in *fpr3*Δ, *fpr4*Δ, and *fpr3*Δ *fpr4*Δ cells (Figure 1B). This result suggests that Fpr3 and Fpr4 have a role in regulating the Cse4 protein level *in vivo*.

To examine whether Fpr3 and Fpr4 regulate Cse4 proteolysis, we performed a protein stability assay (Figure 2). Cse4 was transiently induced from a *GAL1* promoter by the addition of galactose. Then, glucose was added to stop *CSE4* transcription, and cells were collected over 80 min. The level of Cse4 was determined by quantitative Western blotting. As expected, deletion of *FPR3* or *FPR4* moderately stabilized Cse4 protein levels *in vivo* (Figure 2, A and B). The level of the stabilization in *fpr3*Δ cells was higher than that in *fpr4*Δ cells. It has been also previously reported that deletion of *PSH1*, which is an E3 ubiquitin ligase, moderately stabilized the Cse4 protein level (Hewawasam *et al.* 2010; Ranjitkar *et al.* 2010). The level of the stabilization in *fpr3*Δ

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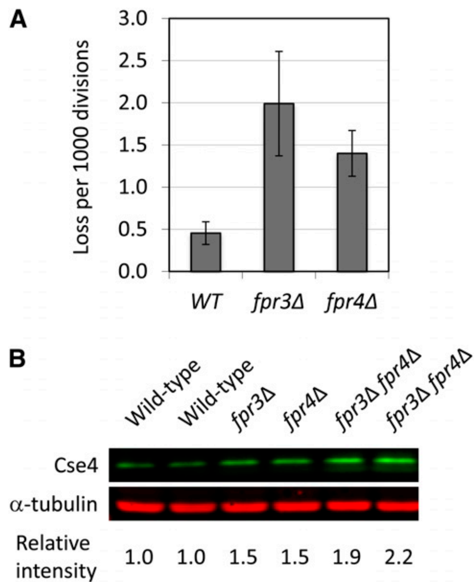

Figure 1 Fpr3 regulates Cse4 protein level *in vivo*. (A) The *fpr3*Δ or *fpr4*Δ strain displays a moderate chromosome missegregation phenotype. Chromosome loss rate in null mutants was determined by half sector analysis, as previously described (Ohkuni *et al.* 2008). Wild-type (Y14): 3 half-sectored colonies/6,421 total colonies; *fpr3*Δ (Y2249, Y2250, and Y2251): 17/8,598; and *fpr4*Δ (Y2252, Y2253, and Y2254): 14/10,105. *P*-value (chi-squared test): WT *vs.* *fpr3*Δ, 0.012; WT *vs.* *fpr4*Δ, 0.072. (B) Increased protein level of Cse4 in *fpr*Δ cells. Equal cell numbers of log phase cells, grown in SRaf medium, were visualized by Western blot analysis with anti-Cse4 and anti-α-tubulin antibodies. Cse4 and α-tubulin protein levels were overlayed and quantitated by the Odyssey Imaging System. Cse4 protein levels were normalized by the amount of α-tubulin. Isogenic yeast strains were wild type (YPH499 and YPH500), *fpr3*Δ (Y2243), *fpr4*Δ (Y2245), and *fpr3*Δ *fpr4*Δ (Y2247 and Y2248).

cells was higher than that in *psh1*Δ cells (Figure 2, C and D and Figure S2). Moreover, double deletion mutant, *fpr3*Δ *psh1*Δ, showed the dramatically increased protein stability of Cse4 (Figure 2, C and D). In all, these results strongly support the idea that Fpr3 and Fpr4 regulate the protein level of Cse4.

FPR3 encodes a peptidylprolyl *cis–trans* isomerase (PPIase) (Benton *et al.* 1994; Shan *et al.* 1994), which is involved in the meiotic recombination checkpoint pathway (Hochwagen *et al.* 2005; Macqueen and Roeder 2009). We next tested whether the peptidylprolyl *cis–trans* isomerase enzymatic activity is important for the Cse4 protein stability. We performed the Cse4 protein stability assay using two Fpr3 catalytic domain point mutants (W363L and F402Y) that have lost PPIase activity (Hochwagen *et al.* 2005). The two PPIase dead mutations caused a stabilization of Cse4 protein level *in vivo*, suggesting that the Fpr3 proline isomerase activity is required for Cse4 protein stability (Figure 3, A and B).

We next aimed to identify the target proline related to the Cse4 protein stability. There are five proline sites in Cse4, which are P53, P98, P100, P134, and P157. We generated yeast strains bearing each mutation (proline to valine) in Cse4 (Table S1) and analyzed the stability of Cse4. Only the P134V mutation has a clear effect on Cse4 stabilization (Figure 3C). This result suggests that P134 may be the target of Fpr3 isomerization.

Psh1 ubiquitinates Cse4 at the following lysine residues in its C terminus: K131, K155, K163, and K172 (Hewawasam *et al.* 2010). As well, Cse4 directly interact with Psh1 via the RING finger domain, which is a hallmark of many E3 ligases (Ranjitkar *et al.* 2010). Because P134 is located close to those ubiquitylation sites, we tested whether deletion of *FPR3* or *FPR4* influences the interaction between Cse4 and Psh1. We performed coimmunoprecipitation and Western blotting using Psh1–myc-tagged strains. Psh1–myc-tagged protein physically interacts with Cse4 in wild-type (WT) cells. Interestingly, deletion of *FPR3* or *FPR4* diminishes the interaction between Cse4 and Psh1–myc (Figure 4, A and B). These data indicate that *FPR3* and *FPR4* regulate the Cse4–Psh1 interaction.

Given that the interaction of Cse4 with Psh1–myc is diminished in *fpr3*Δ cells, we predicted that mutation of P134V in Cse4 also influences the Cse4–Psh1 interaction. As expected, the interaction between cse4–P134V and Psh1–myc was also diminished (Figure 4, C and D). Taken together, these data suggest that structural change between the *cis* and *trans* form of P134 in Cse4 is essential for the Cse4–Psh1 interaction (Figure 4E).

We note that *cse4–P134V* mutants do not show any benomyl sensitivity or chromosome missegregation phenotype (data not shown). These phenotypes are also consistent with that of *psh1*Δ cells (Yuen *et al.* 2007). The phenotypic data also support the idea that Fpr3 regulates the Psh1-dependent Cse4 proteolysis. As Psh1 prevents Cse4 localization at noncentromeric regions (Hewawasam *et al.* 2010; Ranjitkar *et al.* 2010), Fpr3 might also prevent Cse4 interaction with noncentromeric regions. However, we found that Cse4 is accumulated at *CEN* (Figure S3), suggesting that Fpr3 might regulate Cse4 levels at the centromere.

Recently, it has become apparent that the Doa1/Ufd3 (WD repeat protein) is also required for the ubiquitination and proteolysis of Cse4 (Au *et al.* 2013). Doa1 regulates cellular levels of ubiquitin (Zhao *et al.* 2009). They propose that Doa1-mediated ubiquitination of Cse4 might be regulated by other ubiquitin ligases than Psh1 (Au *et al.* 2013). Interestingly, Doa1 is one of the targets of Fpr3 (Collins *et al.* 2007). We have shown that the stabilization level of Cse4 in *fpr3*Δ *psh1*Δ cells is much higher than that in *fpr3*Δ (Figure 2, C and D), implying that Fpr3 might regulate the proteolysis of Cse4 in both Psh1-dependent and Psh1-independent manners.

Loss of Fpr3 causes chromosome missegregation, but not benomyl sensitivity (Figure 1A and Figure S1). Interestingly, a high copy of *FPR3* suppress the Glc7 overexpression lethality and the temperature sensitivity of *ipl1-1* mutant (Ghosh and Cannon 2013). Deletion of Fpr3 showed a synthetic sickness phenotype with *ipl1-2* mutant (data not shown). The opposing Ipl1 (aurora kinase B) and Glc7 (protein phosphatase 1: PP1) activities ensure a bipolar attachment to the spindle (Lampson and Cheeseman 2011). Thus, Fpr3 might

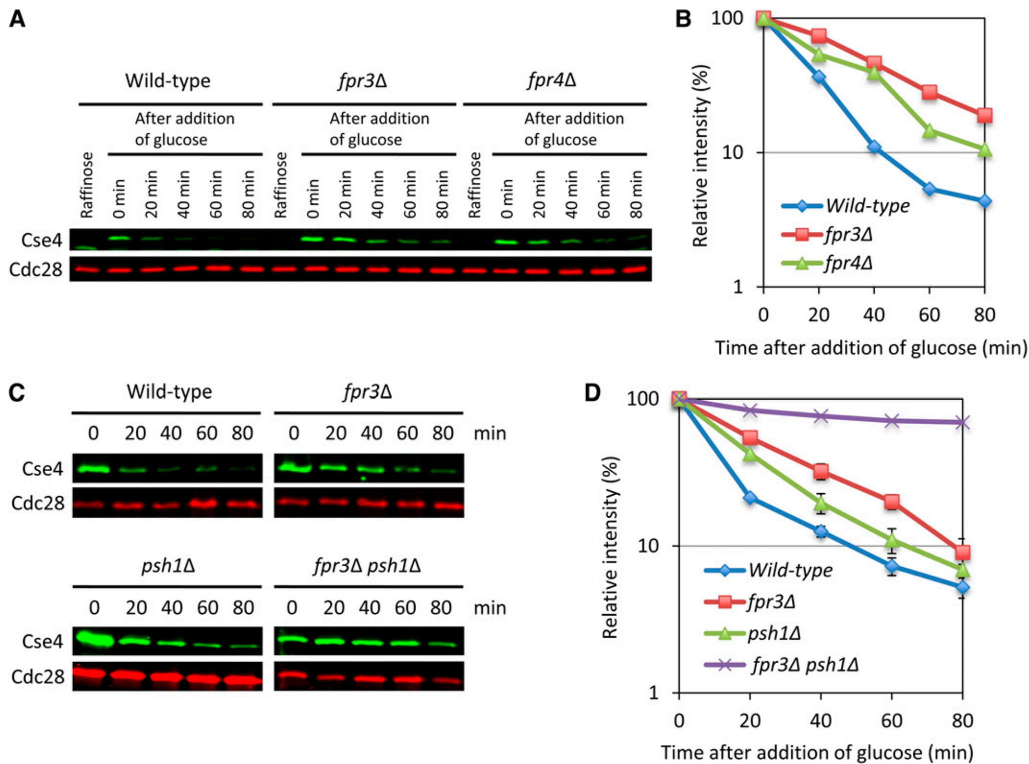

Figure 2 Deletion of *FPR3* stabilizes Cse4 protein level *in vivo*. Cse4 was induced from a *GAL1* promoter by the addition of galactose for 2 hr. Glucose was added and cells were collected at the time point. Equal cell numbers were visualized by Western blot analysis with anti-Cse4, or anti-Cdc28. We used the Odyssey Imaging System to detect and quantify the signals. (In detail, see File S1) (A and B) Isogenic yeast strains were wild type (Y2255), *fpr3*Δ (Y2256), and *fpr4*Δ (Y2257). (C and D) Isogenic yeast strains were wild type (Y2255), *fpr3*Δ (Y2256), *psh1*Δ (Y2258), and *fpr3*Δ *psh1*Δ (Y2340). Error bars represent SE of two independent experiments.

also be involved in another mechanism in the centromere function such as bipolar attachment.

In summary, we characterized the mitotic function of Fpr3, which was identified by our *in vitro* kinetochore assembly system. Fpr3 has a role in Cse4 protein stability. Fpr3 PPIase dead mutants stabilized Cse4 protein, suggesting that the isomerization activity of Fpr3 is necessary for the Cse4 proteolysis. The interaction between Cse4 and Psh1 was

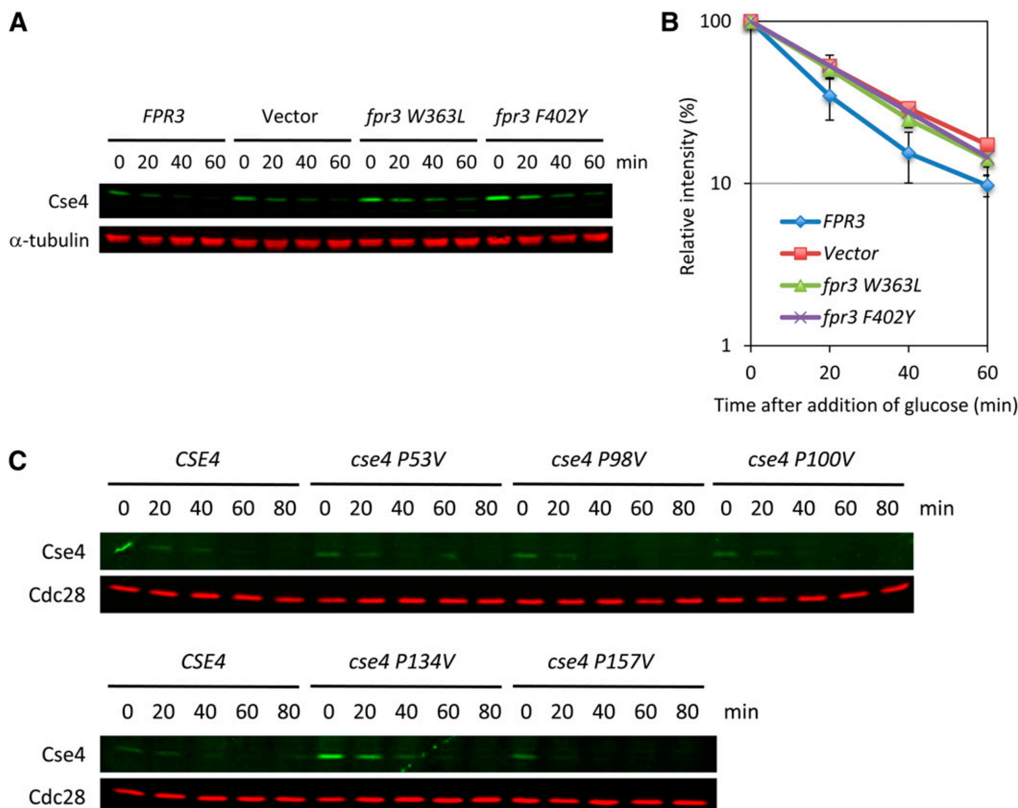

Figure 3 Fpr3 isomerization activity is necessary for the Cse4 proteolysis. (A and B) PPIase dead mutants stabilize the Cse4 protein level *in vivo*. We constructed plasmids harboring PPIase dead mutations (W363L and F402Y) (Table S2). The protein stability assay was performed as described in Figure 2. Isogenic yeast strains were *FPR3* (Y2259), Vector (Y2260), *fpr3 W363L* (Y2261), and *fpr3 F402Y* (Y2262). Error bars represent SE of two or three independent experiments. (C) Mutation of P134 does stabilize the Cse4 protein level *in vivo*. There are five proline sites in Cse4 (P53, P98, P100, P134, and P157). We constructed plasmids harboring proline-to-valine mutation (Table S2). The protein stability assay was performed as described in Figure 2. Isogenic yeast strains were *Cse4* (Y2255), *cse4 P53V* (Y2263), *cse4 P98V* (Y2264), *cse4 P100V* (Y2265), *cse4 P134V* (Y2266), and *cse4 P157V* (Y2267).

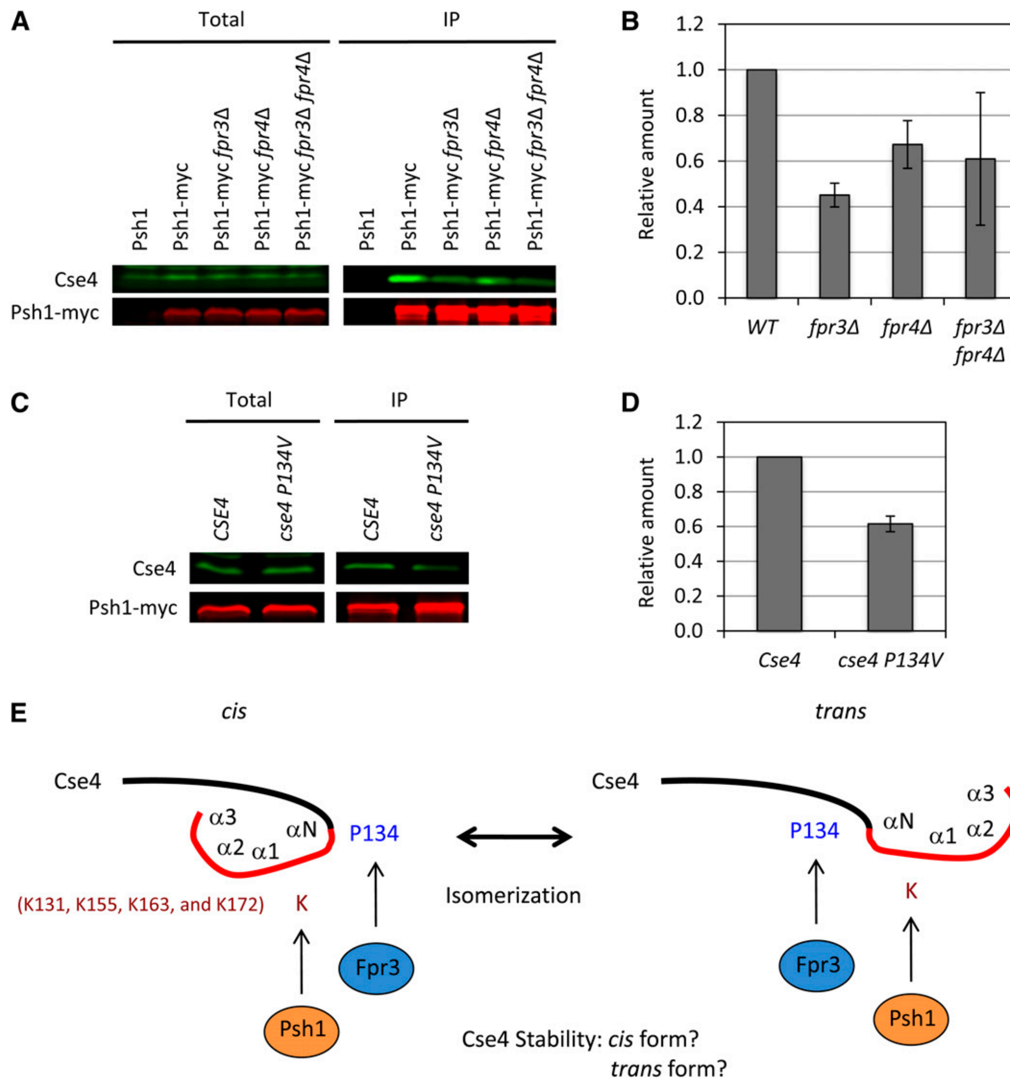

Figure 4 Fpr3 regulates the Cse4–Psh1 interaction. (A and B) Interaction between Cse4 and Psh1 was diminished in *fpr3*Δ cells. The indicated strains were grown to log phase, lysed, and anti-myc immunoprecipitations were performed as previously described (Ohkuni *et al.* 2008). Total and the immunoprecipitated fraction (IP) were subjected to SDS–PAGE, and Western blots were used to detect Cse4 and myc-tagged Psh1. We used the Odyssey Imaging System to detect and quantify the signals. Isogenic yeast strains were untagged (YPH500), Psh1–myc (Y2280), Psh1–myc *fpr3*Δ (Y2281), Psh1–myc *fpr4*Δ (Y2282), and Psh1–myc *fpr3*Δ *fpr4*Δ (Y2283). Error bars represent SE of two independent experiments. Significant difference, $P = 0.0089$ (WT *vs.* *fpr3*Δ). (C and D) P134V mutation in Cse4 diminishes the Psh1 interaction. Anti-myc immunoprecipitation assay and the quantification were performed as described in Figure 4, A and B. Isogenic yeast strains were *Cse4* (Y2284) and *cse4 P134V* (Y2285). Error bars represent SE of two independent experiments. Significant difference, $P = 0.0134$. (E) A model for the role of Fpr3 in the Cse4 proteolysis. Psh1 is the E3 ubiquitin ligase that targets Cse4. Four lysine sites (K131, K155, K163, and K172) were ubiquitinated by Psh1. P134 close to the αN-helix (136–147) (Keith *et al.* 1999) might be the target of Fpr3 isomerization. We propose that the structural change in Cse4 from *cis* to *trans* or from *trans* to *cis* is important for the Cse4 degradation by Psh1. It is not known which form of Cse4 is ubiquitinated. The N-terminal domain (black) and the histone fold domain (red) of the α-N, α-1, α-2, and α-3 helices are indicated (Keith *et al.* 1999).

diminished in the *fpr3*Δ mutant. Furthermore, a mutation on P134, a target of Fpr3 isomerization, reduced the Cse4–Psh1 interaction. Thus, we propose that the structural change between the *cis* and *trans* form in Cse4 is important for the interaction with E3 ubiquitin ligase Psh1 (Figure 4E).

Acknowledgments

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Degradation of Centromeric Histone H3 Variant Cse4 Requires the Fpr3 Peptidyl-prolyl *Cis–Trans* Isomerase

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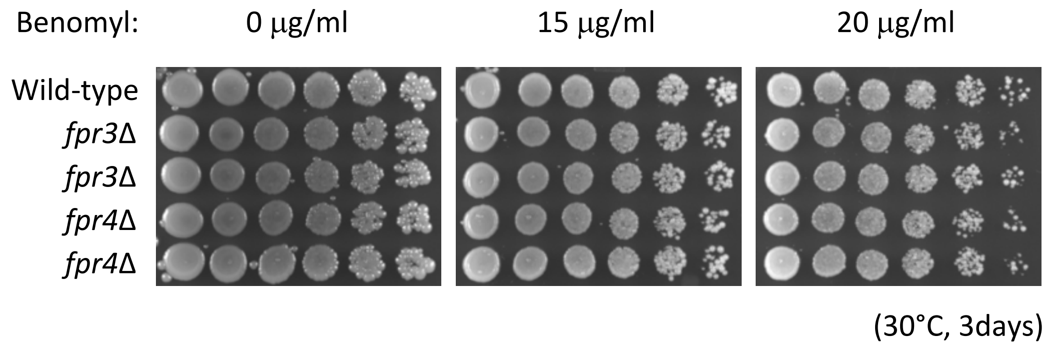

Figure S1 *fpr3*Δ or *fpr4*Δ cells don't show any benomyl sensitivity. Yeast cells were spotted in 5-fold dilutions from 5 x 10^4 cells per spot on YPD plates containing benomyl. The plates were incubated at 30°C for 3 days and photographed. Isogenic yeast strains were wild-type (YPH499), *fpr3*Δ (Y2243 and Y2244), and *fpr4*Δ (Y2245 and Y2246).

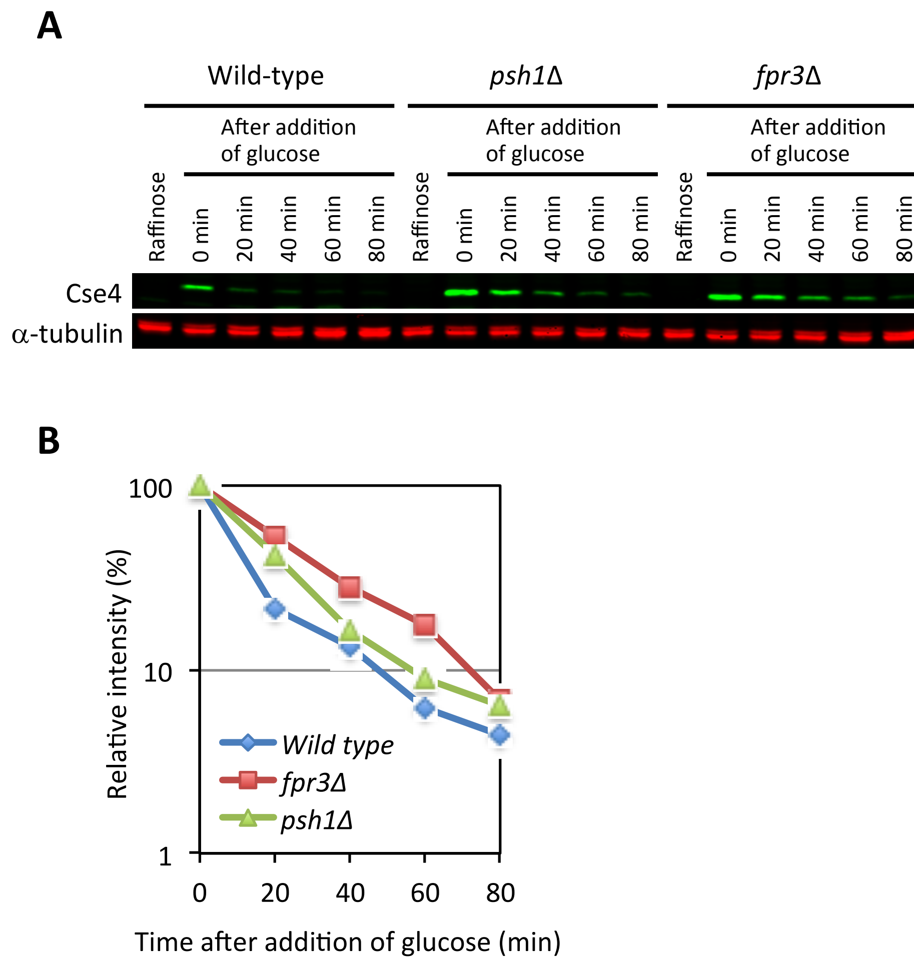

Figure S2 The stabilization level of Cse4 in *fpr3*Δ cells is higher than that in *psh1*Δ cells. The protein stability assay was performed as described in Figure 2. Isogenic yeast strains were wild-type (Y2255), *fpr3*Δ (Y2256), and *psh1*Δ (Y2258).

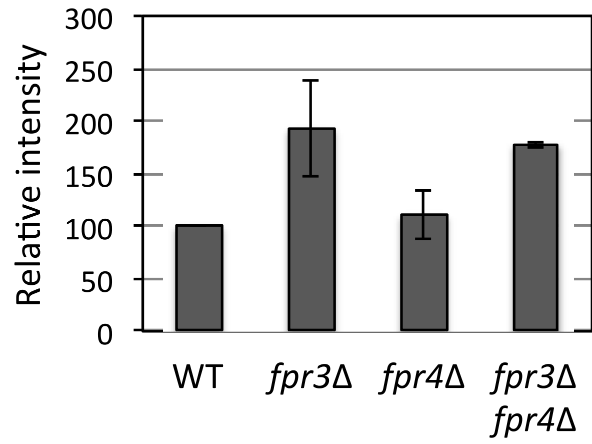

Figure S3 Enhanced *CEN* DNA interaction with Cse4 in *fpr3*Δ mutant. The indicated strains, Cse4-myc (Y2342), Cse4-myc *fpr3*Δ (Y2344), Cse4-myc *fpr4*Δ (Y2345), and Cse4-myc *fpr3*Δ *fpr4*Δ (Y2346), were grown to log phase, lysed, and anti-myc chromatin immunoprecipitation assay was performed as previously described (Ohkuni *et al.* 2008). We used ImageJ to quantify the signals.

File S1

Supplemental methods

Yeast strains and plasmids

Supplemental Table S1 and S2 present the genotype of yeast strains and plasmids used for this study, respectively.

Protein stability assay

Cells were grown in a 2% raffinose synthetic complete medium overnight. Cells were then diluted to an optical density at 600 nm of 0.3 and grown for an additional 3 h at 25°C. A sample was removed as a negative control before the addition of galactose (Raffinose). Galactose was added to the media to a final concentration of 2% to induce expression of Cse4 from the *GAL1* promoter for 2 h at 25°C. Glucose was then added to a final concentration of 5% to stop gene expression, and samples were taken at the indicated time points.

Antibodies

Anti-Cse4 antibody was generated as previously described (Ohkuni *et al.* 2008). Anti-Cdc28 was a gift from Dr. Deshaies's laboratory. Anti-myc (Roche, Indianapolis, IN), anti-HA (Roche, Indianapolis, IN), and anti-tubulin (Serotech, Oxford, UK) antibodies were purchased.

Western blotting and quantitation

Western blotting and quantitation were performed as described previously (Escamilla-Powers and Sears 2007). In brief, protein from equal cell numbers were separated by SDS-PAGE gel and transferred to Immobilon-FL membrane (Millipore, Billerica, MA). Membrane was blocked with Odyssey Blocking buffer (LI-COR Biosciences, Lincoln, NE) after shaking the membrane in PBS for several minutes. Primary and secondary antibodies were diluted in Odyssey Blocking buffer with 0.1% Tween 20. Anti-Cse4 was used at a dilution of 1:1000, and anti-tubulin and anti-Cdc28 were used at 1:5000. Secondary antibodies were used at a dilution of 1:15000. Blots were scanned with a LI-COR Odyssey CLx Imager (Lincoln, NE). Protein levels at each time point were quantitated using Image Studio software version 2.0.

Table S1 ***S. cerevisiae*** **strains used in this study**

Strains	Genotype	Reference
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1*	*(Sikorski and Hieter 1989)*
YPH500	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1*	*(Sikorski and Hieter 1989)*
Y2243	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6*	This study
Y2244	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6*	This study
Y2245	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6*	This study
Y2246	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6*	This study
Y2247	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y2248	*MAT*α *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y14	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11*	P. Hieter
Y2249	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2250	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2251	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr3Δ::His3MX6*	This study
Y2252	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::His3MX6*	This study
Y2253	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::kanMX6*	This study
Y2254	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* CFIII (*CEN3.L.*YPH983) *TRP1 SUP11 fpr4Δ::kanMX6*	This study
Y2255	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2256	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2257	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr4Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2258	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 psh1Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study

Y2340	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6 psh1Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*]	This study
Y2259	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*FPR3*]	This study
Y2260	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416]	This study
Y2261	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*fpr3 W363L*]	This study
Y2262	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 fpr3Δ::kanMX6* [pRS415-*PGAL1-3HA-CSE4*] [pRS416-*fpr3 F402Y*]	This study
Y2263	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P53V*]	This study
Y2264	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P98V*]	This study
Y2265	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P100V*]	This study
Y2266	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P134V*]	This study
Y2267	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [pRS415-*PGAL1-3HA-cse4 P157V*]	This study
Y2280	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1*	This study
Y2281	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr3Δ::kanMX6*	This study
Y2282	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr4Δ::kanMX6*	This study
Y2283	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study
Y2284	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 cse4Δ::TRP1* [pRS415-*CSE4*]	This study
Y2285	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 PSH1-myc:TRP1 cse4Δ::TRP1* [pRS415-*cse4 P134V*]	This study
Y2342	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6*	This study
Y2344	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr3Δ::kanMX6*	This study
Y2345	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr4Δ::kanMX6*	This study

Y2346	*MATα ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 CSE4-myc:His3MX6 fpr3Δ::kanMX6 fpr4Δ::kanMX6*	This study

Table S2 Plasmids used in this study

Plasmid	Relevant characteristics	Reference
B2883	pRS415-*PGAL1-3HA-CSE4*	This study
B2884	pRS416-*FPR3*	This study
B2885	pRS416-*fpr3 W363L*	This study
B2886	pRS416-*fpr3 F402Y*	This study
B2887	pRS415-*CSE4*	This study
B2891	pRS415-*cse4 P134V*	This study
B2893	pRS415-*PGAL1-3HA-cse4 P53V*	This study
B2894	pRS415-*PGAL1-3HA-cse4 P98V*	This study
B2895	pRS415-*PGAL1-3HA-cse4 P100V*	This study
B2896	pRS415-*PGAL1-3HA-cse4 P134V*	This study
B2897	pRS415-*PGAL1-3HA-cse4 P157V*	This study

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