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## Newly identified prion linked to the chromatin-remodeling factor Swi1 in *Saccharomyces cerevisiae*

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### Abstract

SWI/SNF, an evolutionarily conserved ATP-dependent chromatin-remodeling complex, has an important role in transcriptional regulation<sup>1</sup>. In *Saccharomyces cerevisiae*, SWI/SNF regulates the expression of ~6% of total genes through activation or repression<sup>2</sup>. Swi1, a subunit of SWI/SNF, contains an N-terminal region rich in glutamine and asparagine, a notable feature shared by all characterized yeast prions—a group of unique proteins capable of self-perpetuating changes in conformation and function<sup>3</sup>. Here we provide evidence that Swi1 can become a prion, [SWI<sup>+</sup>]. Swi1 aggregates in [SWI<sup>+</sup>] cells but not in nonprion cells. Cells bearing [SWI<sup>+</sup>] show a partial loss-of-function phenotype of SWI/SNF. [SWI<sup>+</sup>] can be eliminated by guanidine hydrochloride treatment, *HSP104* deletion or loss of Swi1. Moreover, we show [SWI<sup>+</sup>] is dominantly and cytoplasmically transmitted. Our findings reveal a novel mechanism of ‘protein-only’ inheritance that results in modification of chromatin-remodeling and, ultimately, global gene regulation.

Yeast prions are epigenetic elements that are transmitted as particular protein conformations<sup>4</sup>, similarly to the mammalian prion that causes infectious diseases<sup>5</sup>. [PSI<sup>+</sup>], [URE3] and [PIN<sup>+</sup>] are three reported prions in *S. cerevisiae*<sup>6-9</sup>, and their protein determinants are Sup35, Ure2 and Rnq1, respectively. They all contain an intrinsic domain with high glutamine and asparagine content essential for prion formation and propagation (Fig. 1a). Yeast prions can be formed and lost at a low spontaneous rate<sup>10</sup>. Overproduction of a prion protein considerably increases *de novo* formation of the corresponding prion<sup>8,11,12</sup>. [PSI<sup>+</sup>] induction by Sup35 overproduction requires a [PSI<sup>+</sup>] inducible factor (Pin<sup>+</sup>), which can be an event such as overproduction of particular glutamine-, asparagine- or glutamine and asparagine-rich proteins, or another prion<sup>13-15</sup>. Notably, several members of the yeast SWI/SNF complex, including Swi1 and Snf5, also contain glutamine and asparagine-rich regions<sup>16</sup> (Fig. 1a), and Swi1 overproduction has been implicated as a Pin<sup>+</sup> factor<sup>9</sup>. SWI/SNF is an evolutionarily conserved, ATP-dependent chromatin-remodeling complex that has a regulatory role in gene expression<sup>17</sup>. In *S. cerevisiae*, the SWI/SNF complex is composed of at least 11 subunits, for a total molecular weight of ~1 MDa<sup>18</sup>. Null mutants of SWI/SNF are viable, but show phenotypes of slow growth, reduced mating-type switching, and inability to utilize nonfermentable carbon sources, such as raffinose, galactose, glycerol and sucrose<sup>19</sup>. In mammals, Ini1, a Snf5 homolog, is essential for embryo viability and tumor suppression<sup>20</sup>. Various mutations affecting the human Ini1 or Snf2 homologs, BRM and BRG-1, are

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associated with many types of malignant tumors<sup>21</sup>. The importance of SWI/SNF function, the notable glutamine and asparagine content of some of its components, and the Pin<sup>+</sup> property of Swi1 led us to investigate whether SWI/SNF function can be subject to prion-like epigenetic regulation.

If Swi1 or Snf5 is a prion protein, it might be more likely to adopt a prion conformation when it functions as a Pin<sup>+</sup>, because prion proteins can interact with each other, and the non-[PSI<sup>+</sup>] yeast prions, [PIN<sup>+</sup>] and [URE3], can function as Pin<sup>+</sup>. Moreover, [PSI<sup>+</sup>] can be easily analyzed in strains such as 74D-694, which contains a nonsense mutation in *ADE1*. Whereas 74D-694 [PSI<sup>+</sup>] cells can grow in media without adenine, [psi<sup>-</sup>] cells cannot. [PSI<sup>+</sup>] colonies appear white in rich media, such as YPD, and [psi<sup>-</sup>] colonies appear red as a result of accumulation of a pigment byproduct. To test whether Swi1 and/or Snf5 are prion proteins, we conducted [PSI<sup>+</sup>]-induction experiments in 74D-694 [psi<sup>-</sup>][pin<sup>-</sup>] cells by co-overexpression of *SWI1* or *SNF5* and *SUP35NMGFP*, a fusion gene encoding the Sup35 prion domain and the green fluorescence protein (GFP). Consistent with a previous report<sup>9</sup>, co-overexpression of *SWI1* and *SUP35NMGFP* allowed [PSI<sup>+</sup>] formation in [pin<sup>-</sup>] cells (Fig. 1b-d). Although the N-terminal region of Snf5 is also rich in glutamine (Fig. 1a), overexpression of *SNF5* did not function as a Pin<sup>+</sup> (Fig. 1b). Therefore, we next focused on examining whether Swi1 is a prion protein.

If Swi1 becomes a prion (termed [SWI<sup>+</sup>]), it might form amyloid-like structure(s), as do other identified prions<sup>22</sup>. Cells bearing [SWI<sup>+</sup>] should show compromised SWI/SNF functions, as the amyloid-form of Swi1 will recruit the nonprion Swi1 isomers and sequester them from their natural contacts. To identify [SWI<sup>+</sup>], we first obtained a large number of Ade<sup>+</sup> colonies ([PSI<sup>+</sup>] candidates) from 74D-694 [psi<sup>-</sup>][pin<sup>-</sup>] cells by co-overexpression of *SWI1* and *SUP35NMGFP*. We then screened for [SWI<sup>+</sup>] candidates by testing individual Ade<sup>+</sup> isolates for phenotypes indicative of compromised SWI/SNF function, specifically, poor growth in raffinose medium (Raf<sup>-</sup>). Among ~400 tested Ade<sup>+</sup> isolates, 32 showed the Raf<sup>-</sup> phenotype. Only two were able to grow on raffinose after 5 mM guanidine hydrochloride treatment (Fig. 2a). This guanidine hydrochloride treatment eliminates all known yeast prions, but does not usually cause nucleic acid mutations<sup>23</sup>. Like SWI/SNF mutants, both candidates grew poorly in galactose (Gal<sup>-</sup>) and glycerol (Gly<sup>-</sup>) (Fig. 2b). However, they showed only a mild defect in using sucrose (Fig. 2b). Unlike SWI/SNF mutants, they did not show any detectable sensitivity to 0.3 M LiCl or 1 M NaCl (Fig. 2b and data not shown). These results suggest that the two [SWI<sup>+</sup>] candidates show an epigenetically conferred, partial loss-of-function SWI/SNF phenotype.

To further investigate whether the observed effect is SWI/SNF specific, we developed a blue-white visual assay using a previously described *lacZ* reporter construct, pLS7, whose expression requires SWI/SNF<sup>24</sup>. A similar visual assay was successfully applied to test the recombinant prion element NMGR<sup>25</sup>. The candidate cells containing pLS7 appeared white on X-gal-sucrose plates but became blue after guanidine hydrochloride treatment (Fig. 2c). Isogenic [swi<sup>-</sup>] cells were blue before and after guanidine hydrochloride treatment. This result provides supporting evidence that the [SWI<sup>+</sup>] candidate cells have compromised SWI/SNF functions.

We next examined the effect of Hsp104, a member of the Clp/Hsp100 family of AAA+ ATPases<sup>26</sup>, on the [SWI<sup>+</sup>] candidates. *HSP104* disruption eliminates all known yeast prions. In contrast, *HSP104* overexpression abolishes [PSI<sup>+</sup>] but not [PIN<sup>+</sup>] or [URE3]<sup>10</sup>. We found that [SWI<sup>+</sup>] candidates became Ade<sup>-</sup> and Raf<sup>+</sup> upon *HSP104* disruption (Fig. 2d and Supplementary Table 1 online) and maintained these phenotypes after reintroduction of a *HSP104* expression plasmid (data not shown). These results demonstrate that, as for [PSI<sup>+</sup>], a transient loss in Hsp104 production eliminates the Raf<sup>-</sup> phenotype; therefore, Hsp104 is

essential for maintaining the  $Raf^-$  trait. We conclude that the  $Raf^-$  phenotype is protein-based and that the  $[SWI^+]$  candidates actually contain two prions:  $[SWI^+]$  and  $[PSI^+]$ .

We also examined the effect of *HSP104* overexpression on  $[SWI^+]$  by transforming  $[PSI^+]$   $[SWI^+]$  cells with *p2HG-HSP104*, a multicopy *HSP104* plasmid with the strong and constitutive *GPD* (glyceraldehyde-3-phosphate dehydrogenase) promoter. All fresh transformants were mixtures of  $Ade^+/Ade^-$  (~36-42%  $Ade^+$ ) and  $Raf^+/Raf^-$  cells (~82-90%  $Raf^-$ ). After a longer time expression, most cells had lost  $[PSI^+]$ , yet they retained the mixed  $Raf^+/Raf^-$  phenotype (~68 - 87%  $Raf^-$ ) (Supplementary Table 2 online). Subsequent removing of *p2HG-HSP104* did not alter their phenotypes. The growth phenotype of one representative  $[psi^-]$  isolate obtained upon *HSP104* overexpression is shown in **Figure 2e,f**. Although some  $[PSI^+]$   $[SWI^+]$  cells lost both prions upon *HSP104* overexpression, all examined  $[PSI^+]$  cells were  $Raf^-$ . Moreover,  $[SWI^+]$  was retained in most  $[psi^-]$  cells (Supplementary Table 2). Thus,  $[SWI^+]$  is tolerant to Hsp104 overproduction.

We obtained several  $[psi^-][SWI^+]$  isolates with stable but guanidine hydrochloride-curable  $Raf^-$  phenotypes after Hsp104 overproduction (Fig. 3a and data not shown). When *pLS7* was introduced into these isolates, they appeared white on X-gal-sucrose plates but blue upon guanidine hydrochloride treatment. However, the isogenic  $[psi^-][swi^-]$  cells were blue in both conditions (Fig. 3b). These data show that  $[SWI^+]$  can exist as a prion independent of  $[PSI^+]$ . Similar to  $[PSI^+][SWI^+]$  cells,  $[psi^-][SWI^+]$  cells were also  $Gly^-$  and  $Gal^-$  (Fig. 3a and Supplementary Fig. 1 online).  $[SWI^+]$  in the  $[psi^-]$  background could be eliminated by *HSP104* disruption (Fig. 3c, Supplementary Table 1 and Supplementary Fig. 1) but not by Hsp104 overproduction (Fig. 3d), suggesting that the partial loss of  $[SWI^+]$  in the  $[PSI^+]$  background upon Hsp104 overproduction is a result of the loss of  $[PSI^+]$ .

Because of their ‘infectious’ nature, all yeast prions identified to date are transmitted cytoplasmically as dominant traits. To test whether this is also the case for  $[SWI^+]$ , we crossed  $[SWI^+]$  cells in both  $[PSI^+]$  and  $[psi^-]$  backgrounds with isogenic  $[swi^-][psi^-]$  cells. The resulting diploids showed the  $Raf^-$  phenotype (Fig. 4a and Supplementary Fig. 2 online), indicating that  $[SWI^+]$  is dominant. We also observed  $[SWI^+]$  dominance when 74D-694  $[SWI^+]$  cells were crossed with isogenic *NMA-SUP35* ( $[pin^-][swi^-]$ ) and *hsp104Δ* cells, or outcrossed with  $[swi^-]$  cells of c10B-H49 and S288C (Supplementary Fig. 2). In all cases,  $[SWI^+]$  diploids could be cured by guanidine hydrochloride treatment (Supplementary Fig. 2). Homologous diploids of *SWI/SNF* null mutants are deficient in sporulation<sup>19</sup>.  $[SWI^+]$  diploids are also poorly sporulated. After repeated attempts, we obtained full spores of seven tetrad sets from 74D-694  $[SWI^+]$  diploids, all of which were  $Raf^-$ , showing that  $[SWI^+]$  is transmitted as a nonmendelian element (Fig. 4b and Supplementary Fig. 3 online). We next used a *kar1* mutant strain to carry out cytoduction experiments, which allow mating partners to mix their cytoplasm without nuclear material transfer<sup>27</sup>.  $[SWI^+]$  in both  $[PSI^+]$  and  $[psi^-]$  backgrounds was successfully cytoduced from a 74D-694 strain to a c10BH49 strain containing *kar1-1*. The c10B-H49 cytoductants of  $[SWI^+]$  grew poorly on raffinose, and this  $Raf^-$  phenotype could be eliminated by guanidine hydrochloride treatment or *HSP104* disruption (Fig. 4c and Supplementary Fig. 4 online). The cytoduction efficiencies of  $[SWI^+][PSI^+]$  and  $[SWI^+][psi^-]$  were about 89% and 49%, respectively (Supplementary Table 3 online). The higher cytoduction rate of  $[SWI^+]$  in the  $[PSI^+]$  background suggests that  $[PSI^+]$  can facilitate  $[SWI^+]$  transmission. From these data, we conclude that  $[SWI^+]$  can be transmitted between cells through a simple cytoplasm mixing, and thus is ‘infectious’.

We next tested whether Swi1 had undergone conformational changes in  $[SWI^+]$  cells. We transformed  $[PSI^+][SWI^+]$  and  $[PSI^+][swi^-]$  cells with *p416TEFSWI1YFP*, a centromere-based plasmid expressing the Swi1-YFP fusion under a weak and constitutive *TEF* (translation elongation factor 1 $\alpha$ ) promoter. We observed that  $[PSI^+][SWI^+]$  cells showed numerous sharp

fluorescent foci, indicative of aggregates, but isogenic [*PSI*<sup>+</sup>][*swi*<sup>-</sup>] cells did not (Fig. 5a). Moreover, [*PSI*<sup>+</sup>][*SWI*<sup>+</sup>] cells treated with guanidine hydrochloride did not show detectable fluorescent foci (Fig. 5a). Similarly, numerous fluorescent foci were observed in [*psi*<sup>-</sup>][*SWI*<sup>+</sup>] cells but not in [*psi*<sup>-</sup>][*swi*<sup>-</sup>] cells or in [*psi*<sup>-</sup>][*SWI*<sup>+</sup>] cells treated with guanidine hydrochloride (Fig. 5b).

We also examined the fluorescence patterns of Swi1-YFP in c10BH49 cytoductants of [*PSI*<sup>+</sup>][*SWI*<sup>+</sup>] and [*psi*<sup>-</sup>][*SWI*<sup>+</sup>]. Like their 74D-694 [*SWI*<sup>+</sup>] donors, [*SWI*<sup>+</sup>] cytoductants containing *p416TEFSWIIYFP* showed fluorescent foci of Swi1-YFP, but isogenic [*swi*<sup>-</sup>] cells or [*SWI*<sup>+</sup>] cells after guanidine hydrochloride treatment or *HSP104* disruption did not (Fig. 5c and data not shown). Moreover, Swi1-YFP aggregates were also observed in [*SWI*<sup>+</sup>] diploids containing *p416TEF-SWIIYFP* but not in the isogenic [*swi*<sup>-</sup>] diploids (Fig. 5d). Our data thus demonstrate that Swi1 exists in altered and aggregated conformational states in [*SWI*<sup>+</sup>] cells. The Swi1-YFP foci seemed to be mainly localized in the cytoplasm, as the signals of Swi1-YFP and Hoechst dye (a DNA-binding dye) generally did not overlap (Fig. 5e). As the 74D-964 [*SWI*<sup>+</sup>] strain described in this study carries a *YFP* tag inframe with the chromosomal *SNF5* (see Methods), we examined the fluorescence pattern of Snf5-YFP in both [*SWI*<sup>+</sup>][*PSI*<sup>+</sup>] and [*SWI*<sup>+</sup>][*psi*<sup>-</sup>] cells containing no plasmids. We did not detect Snf5-YFP fluorescent foci in any of these cells (Fig. 5f).

It has been shown that some nonprion aggregation-prone proteins, such as poly-Q peptides, can form aggregates in yeast<sup>15,28</sup>. Thus, Swi1-YFP aggregation in [*SWI*<sup>+</sup>] cells suggests but does not prove that Swi1 is the protein determinant of [*SWI*<sup>+</sup>]. To obtain further proof, we created a truncated Swi1 (Swi1-C) protein that lacks the first 535 amino acids containing the glutamine and asparagine-rich regions (Fig. 1a). Ectopic expression of both *SWII-C* and the full-length *SWII* were able to fully complement *SWII* deletion in S288C (Fig. 6a, Supplementary Fig. 5 online). This result demonstrates that Swi1-C is sufficient for Swi1 function. Of note, Swi1-C could suppress the Raf<sup>-</sup> phenotype of [*SWI*<sup>+</sup>], whereas the full-length *SWII* could not (Fig. 6b). Both *swi1Δ* and [*SWI*<sup>+</sup>] strains restored the Raf<sup>-</sup> phenotype upon elimination of the *SWII-C* and *SWII* plasmids (Fig. 6c). These results indicate that the ectopically expressed full-length Swi1 was recruited by [*SWI*<sup>+</sup>] aggregates, whereas Swi1-C was not. The fact that Swi1-C is capable of masking the [*SWI*<sup>+</sup>] phenotype strongly supports the notion that Swi1 is the determinant of [*SWI*<sup>+</sup>].

Prion propagation requires a continuous supply of substrate, such that even transient loss of a prion protein results in loss of the corresponding prion. To test whether constitutive expression of *SWII* is required for [*SWI*<sup>+</sup>] propagation, we cytoduced [*SWI*<sup>+</sup>] to *swi1Δ* cells ([*psi*<sup>-</sup>][*pin*<sup>-</sup>]) and then reintroduced a *SWII* expression plasmid. This transient loss of Swi1 production caused the loss of [*SWI*<sup>+</sup>] (Fig. 6d-f); however, transient loss of Snf5 production did not affect [*SWI*<sup>+</sup>] propagation. The *snf5Δ* cytoductants (containing a *SNF5* plasmid) showed a guanidine hydrochloride-curable Raf<sup>-</sup> phenotype, but the isogenic *swi1Δ* cytoductants (containing a *SWII* plasmid) did not (Fig. 6d). Moreover, we observed that Swi1-YFP foci were only in *snf5Δ* cytoductants (Fig. 6e). Among 88 examined *swi1Δ* cytoductants, none retained [*SWI*<sup>+</sup>]. However, [*SWI*<sup>+</sup>] existed in 7 of 13 examined *snf5Δ* cytoductants (Fig. 6f). These results demonstrate that Swi1 is the protein determinant of [*SWI*<sup>+</sup>].

We have identified a novel prion that epigenetically modifies the function of the chromatin-remodeling complex SWI/SNF. Swi1 can adopt altered and heritable conformations without *SWII* overexpression (Supplementary Fig. 6 online), suggesting that [*SWI*<sup>+</sup>] can occur naturally. Although the frequency of [*SWI*<sup>+</sup>] occurrence remains to be determined, the fact that Swi1 is able to spontaneously switch between alternative conformations strongly suggests that prion-mediated 'protein inheritance' can be a regulatory mechanism contributing to global gene expression.

## METHODS

### Yeast strains

The  $[SWI^+]$  prion was initially established in a 74D-694 strain whose chromosomal *SNF5* was fused inframe with *YFP* (*MATa ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112 SNF5YFP::kanMX4 [psi<sup>-</sup>][pin<sup>-</sup>]*). To test  $[SWI^+]$  dominance, we used a nontagged 74D-694 strain (*MATa ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112 [psi<sup>-</sup>][pin<sup>-</sup>]*) for mating to generate diploids and spores. Strain c10B-H49 (*MATa ade2-1 SUQ5 lys1-1 his3-11,15 leu2-3, 112 kar1-1 ura3::KanMX4 [psi<sup>-</sup>][pin<sup>-</sup>]*) was used for cytoductions. We used a S288C strain (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gpa1Δ [psi<sup>-</sup>][pin<sup>-</sup>]*) in mating and for SWI/SNF phenotypic assays. A *swi1Δ* strain (*MATa his3Δ1 lys1 leu2Δ0 met15Δ0 ura3Δ0 swi1::KanMX4 [psi<sup>-</sup>][pin<sup>-</sup>]*) obtained from sporulating a S288C diploid (*SWII/swi1Δ*, ATCC number: 4022812) was used in *SWII* complementation analysis and cytoduction. A S288C *snf5Δ* strain (*MATa his3Δ1 lys1 leu2Δ0 met15Δ0 ura3Δ0 snf5::KanMX4 [psi<sup>-</sup>][pin<sup>-</sup>]*, ATCC number: 4007175) was used in cytoduction. Diploids from outcrossing of 74D-694 to c10B-H49 or c10B-H49 to S288C were formed occasionally in cytoduction and partially characterized.

Yeast culture and general manipulations were done as previously described<sup>29</sup>. For testing SWI/SNF phenotypes, 1 μg/ml antimycin A from *Streptomyces sp.* (Sigma) was supplemented to media containing 2% of the following nonfermentable carbon sources: raffinose, sucrose, or galactose.

### Plasmids

The *SNF5* ORF was amplified by PCR using plasmid *pLY14* (ref. 30) as a template. The 3.5-kb PCR product was initially cloned into *p416GPD* then subcloned into the *Bam*HI and *Xho*I sites in *p426GPD* to obtain *p426GDPSNF5*. The *SWII* ORF was amplified by PCR from 74D-694 genomic DNA. The 4.0-kb PCR fragment was first ligated to the *Spe*I and *Xho*I sites in *p416TEF* then subcloned into the same sites in *p426GPD* to obtain *p426GPDSWII*. We created plasmid *p416TEFSWIIYFP* by fusing the 4.0-kb *SWII* PCR product inframe to the 5' terminus of the *YFP* gene using the *Spe*I and *Sma*I sites in *p416TEFYFP*. To create *p426GPDGFP*, we cloned the 0.75-kb *GFP* fragment into *p416TEF* through *Nhe*I and *Sac*I and then subcloned it into *p426GPD* through *Spe*I and *Xho*I sites. To create the *SWII-C* allele, we amplified the 2.3-kb fragment of *SWII* lacking the 5' region encoding the first 535 amino acids from *p416TEFSWIIYFP* by PCR. The PCR products were ligated to *p416TEF* through *Spe*I and *Xho*I sites to produce the plasmid *p416TEFSWII-C*. All constructs were confirmed by DNA sequencing.

The reporter plasmid pLS7 (ref. 24) was provided by M. Carlson. Plasmids *p416CUP1RNQ1GFP*, *pRS313CUP1NMGFP*, *p2HGHSP104* and *p2HGHG* have been described previously<sup>29</sup>. The *HSP104* disruption plasmid was a gift from S. Lindquist.

### Cytoduction

We carried out cytoductions by co-incubating donor and recipient cells overnight at 30 °C and then spreading the incubation mixture onto selective media for cytoductants. Individual isolates that had recipient mating types and that lacked donor's chromosomal markers and contained recipient auxotrophic markers were scored as cytoductants. To cytoduce 74D-694 (*MATa*)  $[SWI^+]$  in both  $[PSI^+]$  and  $[psi^-]$  backgrounds to c10B-H49 (*MATa*, *kar1-1*), we integrated the donors with a *URA3* marker by transforming the linearized plasmid *pRS306* after *Stu*I digestion. The resulting Ura<sup>+</sup> transformants were further transformed with *p425GALI* (Leu<sup>+</sup>) and used as donors. Isolates that were Leu<sup>+</sup>, Trp<sup>+</sup>, Ura<sup>-</sup> and *MATa* were scored as cytoductants. To cytoduce  $[SWI^+]$  to S288C *swi1Δ* and *snf5Δ* cells, we incubated  $[SWI^+]$  cells of c10B-H49



(*MAT $\alpha$* , *kar1-1*) containing *p416TEFSWII-C* with *swi1 $\Delta$*  and *snf5 $\Delta$*  cells (S288C *MAT $\alpha$* ) with an integrated *HIS3* marker overnight at 30°C. Cytoductants were selected on synthetic complete medium that lacked uracil and histidine. Isolates that were Ura<sup>+</sup>, His<sup>+</sup>, Met<sup>-</sup>, Lys<sup>+</sup> and *MAT $\alpha$*  were scored as cytoductants. We eliminated the *p416TEFSWII-C* plasmid from the cytoductants by growing them on 5-fluoroorotic acid (5-FOA) containing media before 5 mM guanidine hydrochloride treatment, then we transformed *p416TEFSWII* to *swi1 $\Delta$*  cytoductants, *pLY14* (*SNF5* expression plasmid) to *snf5 $\Delta$*  cytoductants, or *p416TEFSWIIYFP* to both *swi1 $\Delta$*  and *snf5 $\Delta$*  transformants. The resulting transformants were subsequently examined for their abilities to grow on media containing raffinose as the sole carbon source and to form Swi1-YFP aggregates. A corresponding empty vector was included as control for each transformation experiment.

### Nuclear localization

We used Hoechst 33342 dye (Invitrogen) to stain DNA according to the manufacturer's instructions.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

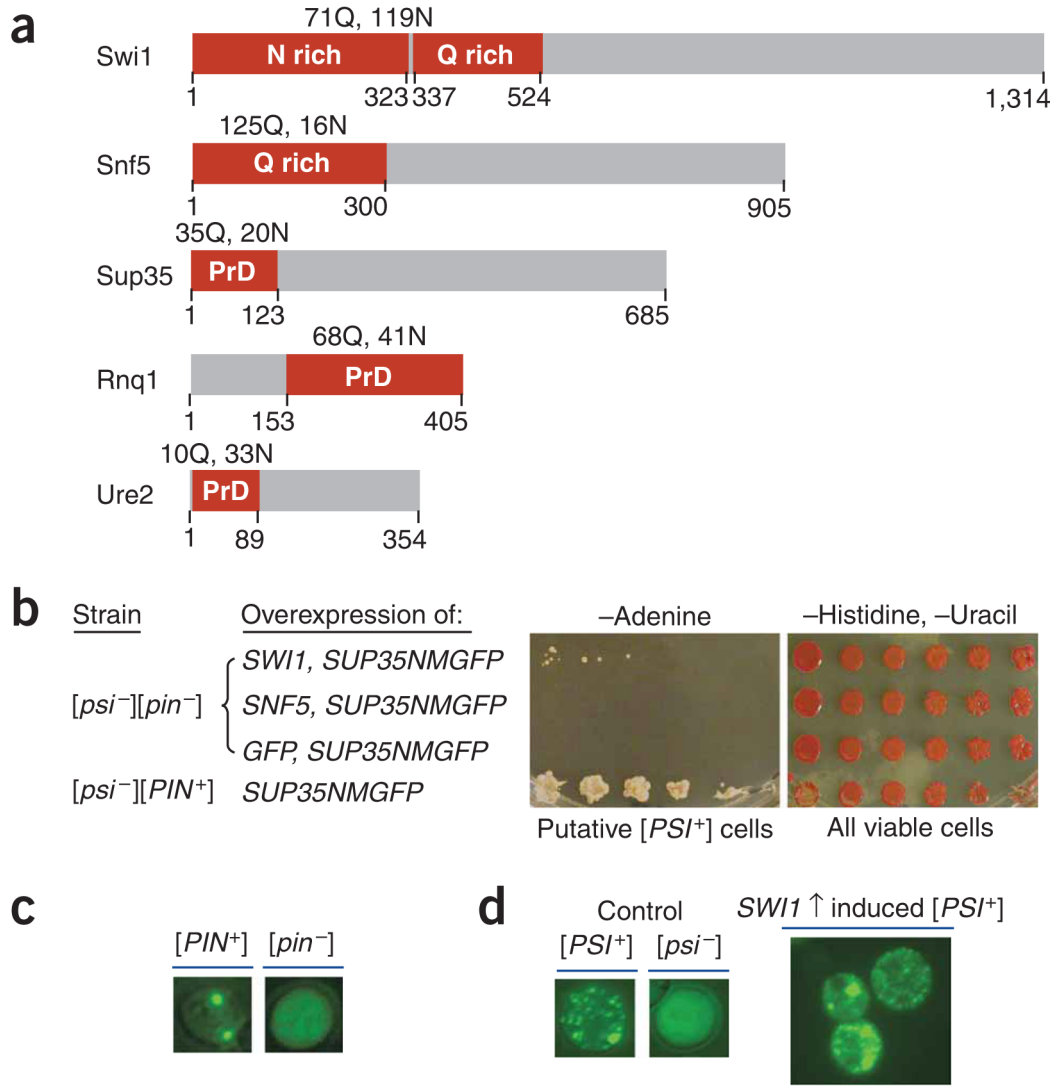
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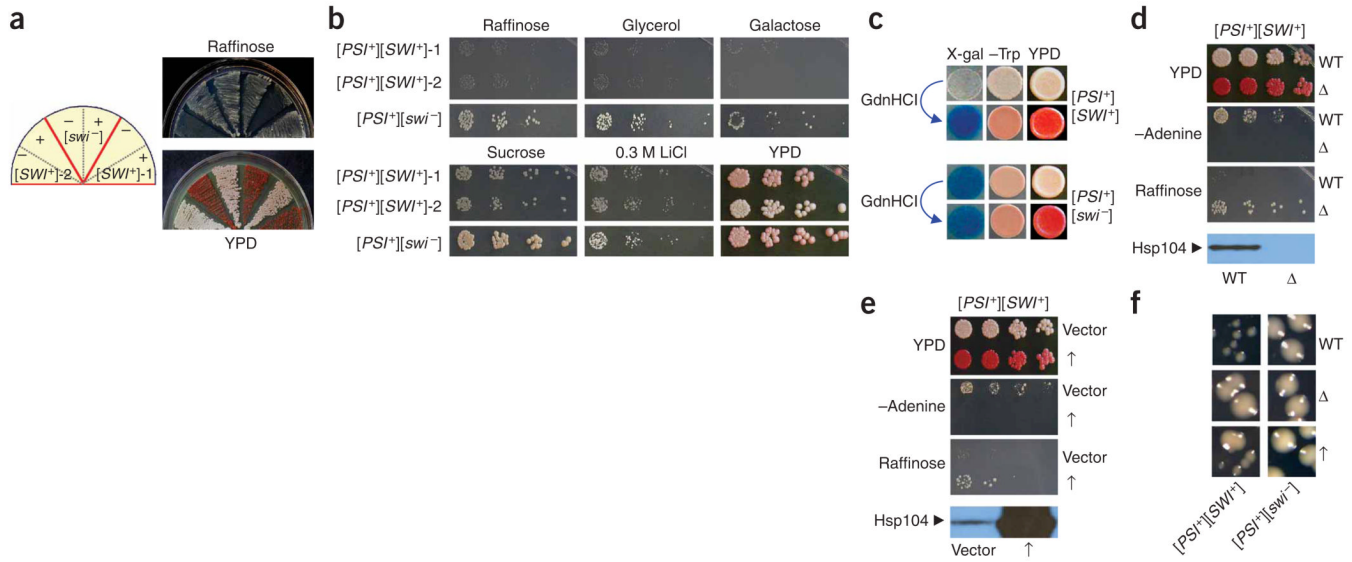


**Figure 1.**

Overexpression of *SWI1* but not *SNF5* functions as *Pin*<sup>+</sup>. **(a)** Diagrams of *Swi1* and *Snf5* with their glutamine (Q) and asparagine (N) rich regions shown (red boxes). The prion domains (PrDs) of *Sup35*, *Rnq1* and *Ure2* are also indicated (red boxes). **(b)** Overexpression of *SWI1* can substitute for [*PIN*<sup>+</sup>] to promote [*PSI*<sup>+</sup>] formation. We grew 74D-694 cells [*psi*<sup>-</sup>][*pin*<sup>-</sup>] containing *pRS313CUP1NMGFP* and *p426GPDSWI1*, *pRS313CUP1NMGFP* and *p426GPDSNF5*, or *pRS313CUP1NMGFP* and *p426GPDGFP* to early-log phase and induced them for *NMGFP* expression by addition of *CuSO*<sub>4</sub> to 100 μM. After 24-h induction, cells were spotted onto the indicated plates with a fivefold serial dilution. Pictures were taken after 5-d incubation for cells on synthetic complete medium lacking histidine and uracil (-Histidine, -Uracil) or after 9 d for cells on medium lacking adenine (-Adenine). **(c)** Fluorescence microscopy assay of *Rnq1* conformational status. Before conducting experiments shown in panel **b**, we confirmed the *Rnq1* conformational status by transforming with *p416CUP1RNQ1GFP*, inducing for 4 h with 100 μM *CuSO*<sub>4</sub> and analyzing by fluorescence microscopy. **(d)** *Sup35* is aggregated in *Ade*<sup>+</sup> cells obtained by *SWI1* and *SUP35NMGFP* co-overexpression. After eliminating *p426GPDSWI1* and *pRS313CUP1NMGFP*, we transformed *Ade*<sup>+</sup> isolates from panel **b** with *p416CUP1NMGFP* and analyzed them by fluorescence

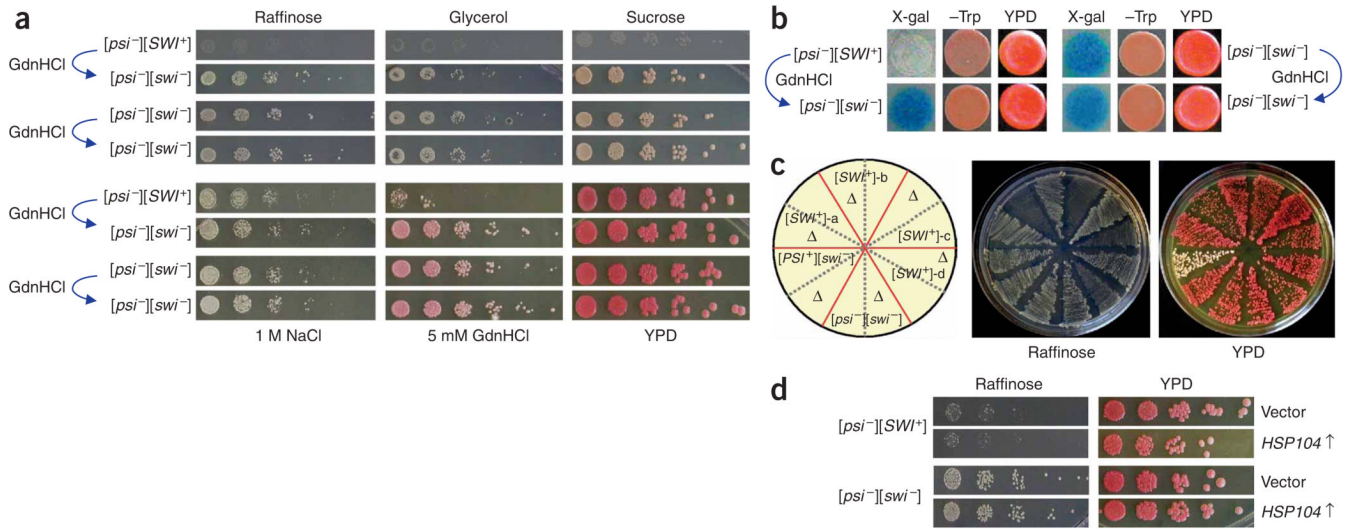


microscopy to confirm their [*PSI*<sup>+</sup>] status. Shown here are fluorescence patterns of a representative [*PSI*<sup>+</sup>] isolate and isogenic [*PSI*<sup>+</sup>] and [*psi*<sup>-</sup>] controls.

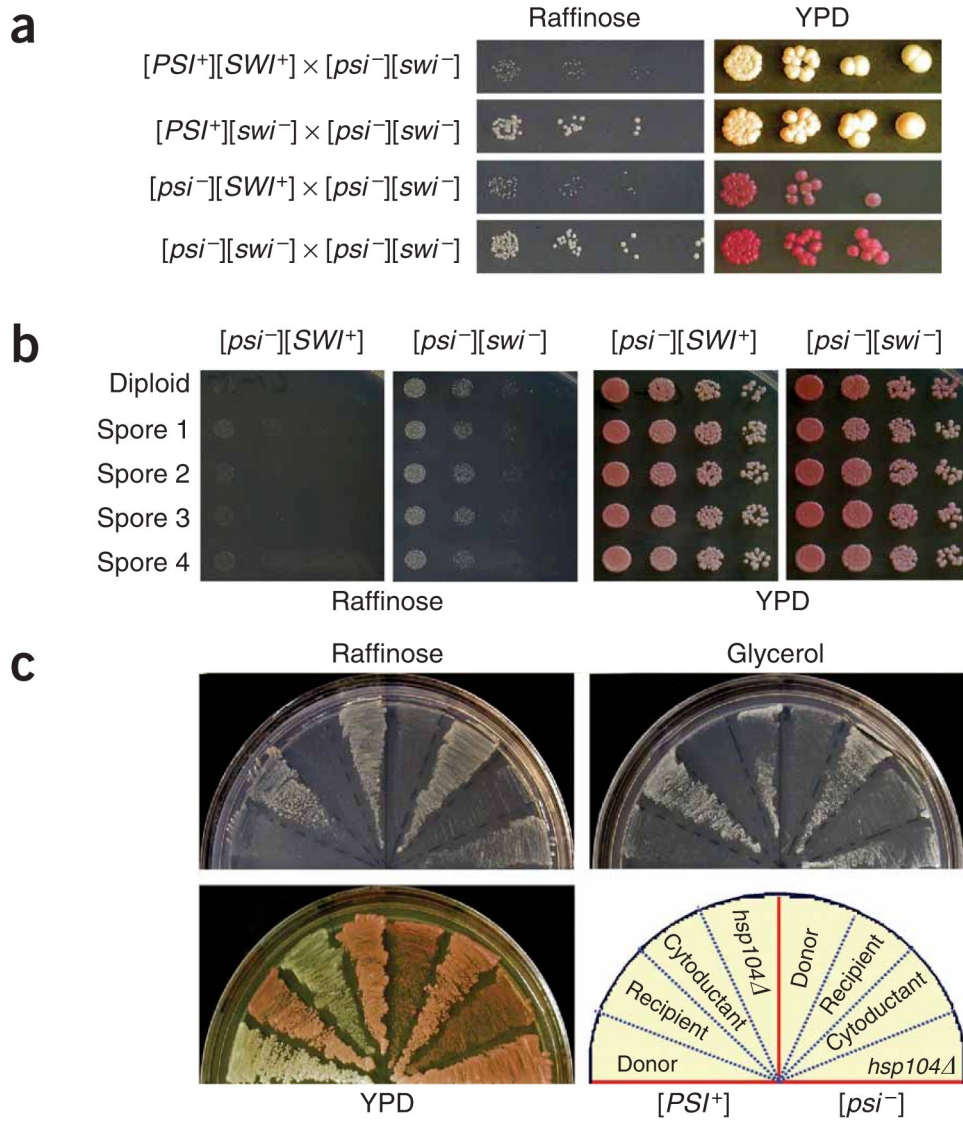


**Figure 2.**

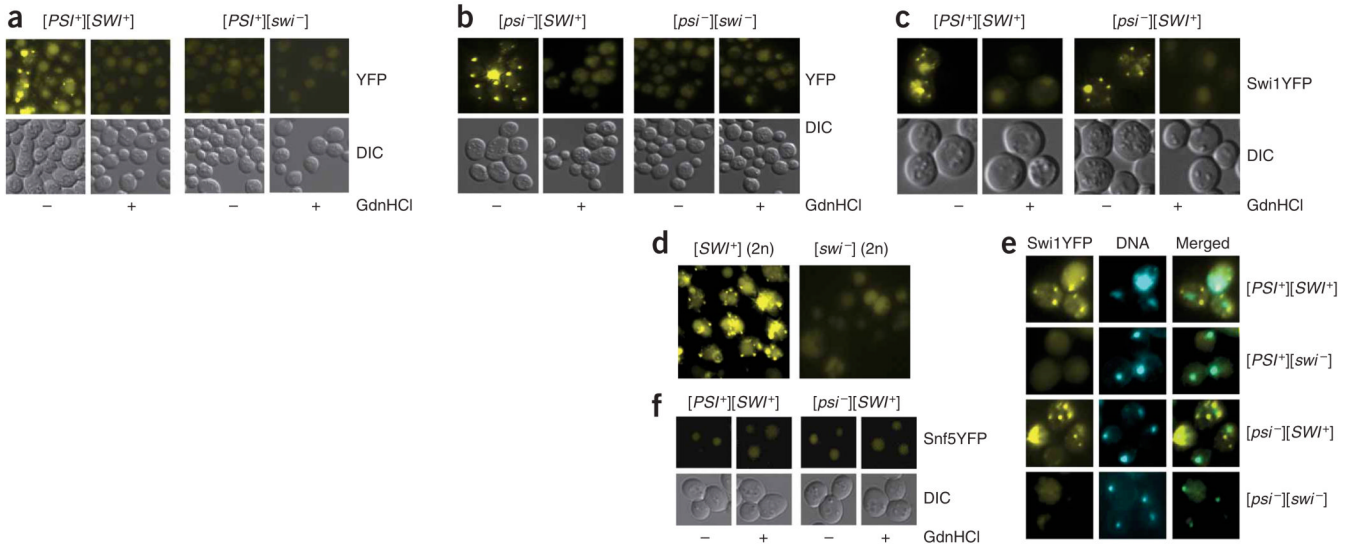
Isolation and characterization of [SWI<sup>+</sup>] candidates. **(a)** [SWI<sup>+</sup>]-1 and [SWI<sup>+</sup>]-2, two [SWI<sup>+</sup>] candidates isolated from [PSI<sup>+</sup>] cells upon co-overexpression of *SUP35NMGFP* and *SWI1*, showed a guanidine hydrochloride-curable Raf<sup>-</sup> phenotype. Shown are cell streaks of the candidates before (-) and after (+) guanidine hydrochloride (GdnHCl) treatment on indicated media. Note that both of the plasmids, *pRS313CUP1NMGFP* and *p426GPD-SWI1*, were eliminated before 5 mM guanidine hydrochloride treatment. **(b)** Phenotypic assays of the [PSI<sup>+</sup>][SWI<sup>+</sup>] candidates. [SWI<sup>+</sup>] candidates and [SWI<sup>-</sup>] control were grown in YPD to mid-log phase and then spotted to the indicated media with a fivefold serial dilution. **(c)** [SWI<sup>+</sup>]-2 (upper) and [SWI<sup>-</sup>] (lower) cells containing *pLS7* plasmid (Trp<sup>+</sup>), a SWI/SNF *lacZ* reporter controlled by a chimeric promoter composed of the SWI/SNF regulatory sequence of *SUC2* promoter and the core promoter of *LEU2* (ref. 24), were spotted onto +sucrose -tryptophan synthetic complete media + 20 μg/ml X-gal plates (X-gal); +glucose -tryptophan synthetic complete medium plates (-Trp); and YPD (YPD) plates. **(d)** *HSP104* deletion eliminated [PSI<sup>+</sup>] and the Raf<sup>-</sup> phenotype. [SWI<sup>+</sup>]-2 cells with (Δ) or without (WT) *HSP104* disruption were assayed on indicated media. **(e)** The effect of *HSP104* overexpression on Raf<sup>-</sup> phenotype. [SWI<sup>+</sup>]-2 cells containing *p2HGHSP104* (↑) or *p2HG* (vector) were grown in synthetic complete liquid media lacking histidine and then spotted to the indicated media. *HSP104* disruption and overexpression were confirmed by immunoblot analysis using a polyclonal Hsp104 antibody. **(f)** Enlarged images showing the colony sizes of [PSI<sup>+</sup>][SWI<sup>+</sup>] and [PSI<sup>+</sup>][SWI<sup>-</sup>] cells after *HSP104* deletion (Δ), overexpression (↑) or no treatment (WT). A [PSI<sup>+</sup>] isolate with Raf<sup>+</sup> phenotype obtained under identical conditions for [SWI<sup>+</sup>] candidate isolation was used as the nonprion ([SWI<sup>-</sup>]) control in all experiments described in this figure.



**Figure 3.** *[SWI<sup>+</sup>]* can exist independently from *[PSI<sup>+</sup>]*. **(a)** Phenotypic analysis of a representative *[psi<sup>-</sup>][SWI<sup>+</sup>]* isolate derived from *[PSI<sup>+</sup>][SWI<sup>+</sup>]-2* upon Hsp104 overproduction. After eliminating the *HSP104* overexpression plasmid, *[psi<sup>-</sup>][SWI<sup>+</sup>]* cells treated with or without 5 mM guanidine hydrochloride were spotted onto indicated plates with a fivefold serial dilution. Pictures were taken after 5-d incubation at 30 °C. **(b)** Isogenic *[psi<sup>-</sup>][SWI<sup>+</sup>]* and *[psi<sup>-</sup>][swi<sup>-</sup>]* cells containing *pLS7* were treated with or without 5 mM guanidine hydrochloride and were grown in -tryptophan synthetic complete liquid media to mid-log phase before spotting to +sucrose -tryptophan media + 20 µg/ml X-gal (X-gal), +glucose -tryptophan media (-Trp) and YPD (YPD) plates. **(c)** *HSP104* disruption eliminates *[SWI<sup>+</sup>]* in *[psi<sup>-</sup>][SWI<sup>+</sup>]* isolates. Shown are cell streaks of four isolates on YPD and raffinose plates before and after *HSP104* gene disruption. Δ, *HSP104* disruption. **(d)** The *[SWI<sup>+</sup>]* prion is tolerant to Hsp104 overproduction. Isogenic *[psi<sup>-</sup>][SWI<sup>+</sup>]* and *[psi<sup>-</sup>][swi<sup>-</sup>]* cells containing *p2HG* (vector) or *p2HG<sup>HSP104</sup>* (*HSP104*↑) were cultured and spotted to the indicated plates.



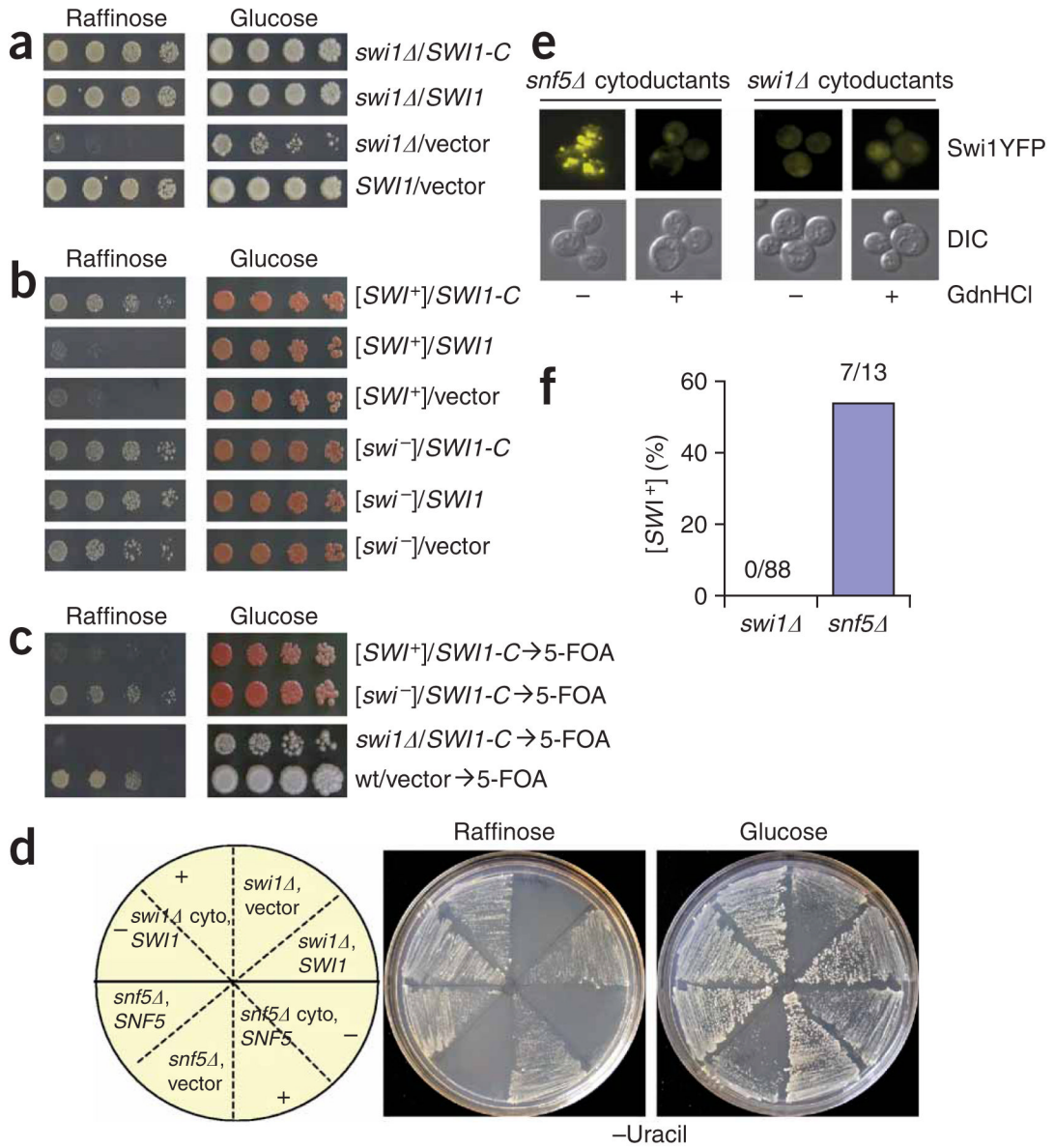
**Figure 4.**  $[SWI^+]$  is dominantly and cytoplasmically inherited. **(a)**  $[SWI^+]$  diploids show  $Raf^+$  phenotype. Diploid cells derived from the indicated crosses were grown in YPD to mid-log phase and spotted onto a raffinose or YPD plate with a fivefold serial dilution. **(b)** All meiotic progenies of  $[SWI^+]$  show the  $Raf^+$  phenotype. Shown are representative phenotypic assays of a full set of spores derived from isogenic  $[SWI^+]$  or  $[SWI^-]$  diploids. **(c)**  $[SWI^+]$  cytoductants of c10B-H49 showed the  $Raf^+$  phenotype that could be eliminated by  $hsp104\Delta$ . Shown are donors, 74D-694  $[PSI^+][SWI^+]$  (left) or  $[psi^-][SWI^+]$  (right); recipient, c10B-H49  $[psi^-][SWI^-]$ ; cytoductants,  $[PSI^+][SWI^+]$  (left) or  $[psi^-][SWI^+]$  (right); and the  $hsp104\Delta$  derivatives of the corresponding cytoductants.



**Figure 5.**

Swi1 but not Snf5 exists in distinct conformational states in  $[SWI^+]$  and  $[swi^-]$  cells. **(a,b)** Swi1-YFP was aggregated in  $[PSI^+][SWI^+]$  **(a)** and  $[psi^-][SWI^+]$  **(b)** but not in isogenic  $[swi^-]$  cells or  $[SWI^+]$  cells after guanidine hydrochloride treatment. Cells as indicated were treated with (+) or without (-) 5 mM guanidine hydrochloride. **(c)** c10B-H49 cytoductants of  $[SWI^+]$  showed guanidine hydrochloride-curable fluorescent foci of Swi1-YFP. **(d)** Swi1-YFP was aggregated in the 74D-694  $[psi^-][SWI^+]$  diploids but not in  $[psi^-][swi^-]$  diploids. **(e)** Swi1-YFP aggregates were mainly localized in cytoplasm. We stained 74D-694  $[SWI^+]$  cells in both  $[PSI^+]$  and  $[psi^-]$  backgrounds with Hoechst dye and analyzed them as described in Methods. **(f)** Endogenous Snf5-YFP is not aggregated in  $[SWI^+]$  or  $[swi^-]$  cells. Cells were treated with (+) or without (-) 5 mM guanidine hydrochloride and analyzed under a fluorescence microscope. All cells were transformed with  $p416TEFSWI1YFP$  and analyzed by fluorescence microscopy assays after growth in synthetic complete medium lacking uracil to mid-log phase.





**Figure 6.** Swi1 is the protein determinant of [SWI<sup>+</sup>]. **(a)** The C domain is sufficient for Swi1 function. Shown are S288C *swi1Δ* cells containing *p416TEFSWI1-C* (SWI1-C), *p416TEFSWI1* (SWI1) or *p416TEF* (vector). Wild-type S288C with *p416TEF* was included as a control. **(b)** SWI1-C, but not the full-length SWI1, was able to ‘complement’ the Raf<sup>-</sup> phenotype of [SWI<sup>+</sup>]. Cells of 74D-694 [*PSI*<sup>+</sup>][SWI<sup>+</sup>] and [*PSI*<sup>+</sup>][*swi*<sup>-</sup>] containing *p416TEFSWI1-C* (SWI1-C), *p416TEFSWI1* (SWI1) or *p416TEF* (vector) were examined for growth on the indicated plates. **(c)** Swi1-C was able to phenotypically ‘mask’ but not cure [SWI<sup>+</sup>]. After plasmid removal, [SWI<sup>+</sup>] and *swi1Δ* cells were re-assayed as described in **a** and **b**. **(d)** Swi1 is required for [SWI<sup>+</sup>] propagation. [SWI<sup>+</sup>] was cytoduced to isogenic *swi1Δ* and *snf5Δ* cells, and the resulting cytoductants were transformed with a SWI1 expression plasmid for *swi1Δ* cells (*swi1Δ* cyto, SWI1) or SNF5 for *snf5Δ* cells (*snf5Δ* cyto, SNF5). Isogenic *swi1Δ* and *snf5Δ* cells were also included as controls. Cell streaks of each representative transformant before (-) or after (+) 5 mM guanidine hydrochloride treatment are shown on the indicated plates. **(e)** Swi1-YFP is

aggregated in the *snf5Δ* but not in the *swi1Δ* cytoductants after [*SWI*<sup>+</sup>] cytoduction. Cytoductants of *swi1Δ* and *snf5Δ* were treated with (+) or without (-) 5 mM guanidine hydrochloride followed by transformation of *p416TEF-SWI1YFP* and analysis by fluorescence microscopy. **(f)** Summary of the [*SWI*<sup>+</sup>] cytoduction experiments. Shown are the results of three independent experiments.