A Small, Glutamine-Free Domain Propagates the $[SWI^+]$ Prion in Budding Yeast[▽]

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Yeast prions are self-propagating protein conformations that transmit heritable phenotypes in an epigenetic manner. The recently identified yeast prion $[SWI^+]$ is an alternative conformation of Swi1, a component of the evolutionarily conserved SWI/SNF chromatin-remodeling complex. Formation of the $[SWI^+]$ prion results in a partial loss-of-function phenotype for Swi1. The amino-terminal region of Swi1 is dispensable for its normal function but is required for $[SWI^+]$ formation and propagation; however, the precise prion domain (PrD) of Swi1 has not been elucidated. Here, we define the minimal Swi1 PrD as the first 37 amino acids of the protein. This region is extremely asparagine rich but, unexpectedly, contains no glutamine residues. This unusually small prion domain is sufficient for aggregation, propagation, and transmission of the $[SWI^+]$ prion. Because of its unusual size and composition, the Swi1 prion domain defined here has important implications for describing and identifying novel prions.

Prion proteins are misfolded, infectious conformers of native host proteins. A unique feature of prions is their ability to self-perpetuate by recruiting soluble, correctly folded protein isomers and inducing them to adopt the prion conformation (38). The mammalian prion (PrP^{Sc}), the pathogen responsible for a group of transmissible neurodegenerative diseases known as prion diseases, is composed of misfolded conformers of the host protein PrP^C (39, 56). Yeast prions are formed from native yeast proteins that adopt misfolded conformations and are transmitted as cytoplasmic traits during mating and budding (8, 52, 53, 58). Almost all yeast prions are composed of amyloid aggregates, which recruit soluble protein isomers into insoluble aggregates, which then assemble into fibrils (3, 42, 47, 57). Fragmentation of these fibrils produces prion “seeds,” which are transmitted in the cytoplasm, accounting for the heritable and infectious nature of yeast prions (11, 17, 19, 22).

While PrP is the only known mammalian prion protein, multiple yeast prions have been identified. $[PSI^+]$ (9) and $[URE3]$ (23) were the first recognized prions in yeast and represent the prion forms of Sup35 and Ure2, respectively (56). Subsequent work has led to the identification of $[PIN^+]$, $[\beta]$, $[SWI^+]$, $[OCT^+]$, $[MOT3^+]$, $[GAR^+]$, and, most recently, $[NSI^+]$ and $[ISP^+]$ (1, 5, 13, 15, 32, 40, 41, 44, 48). One of the common features shared by almost all yeast prion proteins is the modular nature of the protein. Most yeast prion proteins can be divided into two major regions: a functional domain, which is sufficient for native protein activity, and a transferrable prion domain (PrD), which is sufficient for formation and propagation of the prion (24, 48). Interestingly, two reports suggest

that the PrDs of Sup35 and Ure2 may subtly affect protein activity (45, 54), although this phenomenon has not been described for other known yeast prions. In general, yeast PrDs are highly enriched in glutamine (Q) and asparagine (N) residues and have very few charged or hydrophobic residues (1, 10, 43). Several lines of evidence suggest that Q and N contribute similarly to the formation of amyloid fibers: aggregates formed from purified poly(Q), poly(N), or the Q/N-rich Sup35 PrD form analogous polar-zipper structures (34); both Q and N can form amide stacks, a strong stabilizing force for amyloid structure (30, 33); and compositional analysis of known yeast PrDs suggests similar propensities for Q and N in prion-forming domains (51). Furthermore, screening the yeast proteome for Q/N-rich proteins has directly resulted in the discovery of several known yeast prion proteins and has generated a list of multiple promising candidates (1, 26).

Swi1 was originally identified as a yeast prion candidate due to its high Q/N content, as well as its ability to promote $[PSI^+]$ formation (12, 15). Swi1 is a component of the multisubunit SWI/SNF chromatin-remodeling complex, which is responsible for the transcriptional regulation of approximately 6% of the yeast genome (36, 49). Deletion of SWI/SNF components results in defects in the mating type switch and in the metabolism of nonglucose carbon sources (28, 29). Formation of $[SWI^+]$ mimics knockdown of Swi1, which results in phenotypes that include poor sporulation and a reduced ability to grow on raffinose as a sole carbon source (15). Previous work from our laboratory has shown that these phenotypes are most likely attributable to the amino-terminal Q/N-rich region of Swi1 for several reasons: first, deletion of the first 523 amino acids of the protein has no significant effect on carbon source usage, a readily assayable trait regulated by Swi1 (15); second, a peptide comprising the first 327 amino acids of Swi1 forms fibrils *in vitro* that can induce $[SWI^+]$ in wild-type cells (14); and finally, deletion of the genomic sequence coding for the Swi1 amino terminus results in loss of $[SWI^+]$ (14). Based on these data, we

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hypothesized that the minimal Swi1 PrD lay within the first 327 amino acids of the Swi1 protein.

We show here that a small region of 37 amino acid residues at the extreme amino terminus of Swi1 is sufficient for maintenance, propagation, and transmission of the $[SWT^+]$ prion. Swi1₁₋₃₇ decorates $[SWT^+]$ aggregates formed from full-length Swi1 and maintains its aggregated form when Swi1 is eliminated. Aggregated Swi1₁₋₃₇ can transmit the prion fold back to full-length Swi1, both in the same cell, by coexpression and to endogenous protein in a wild-type background by cytoplasmic mixing. Finally, Swi1₁₋₃₇-transmitted $[SWT^+]$ is curable by treatment with millimolar concentrations of guanidine hydrochloride (GdnHCl). These data strongly indicate that Swi1₁₋₃₇ represents the minimal Swi1 prion domain.

MATERIALS AND METHODS

Yeast strains and media. All yeast strains used in this study were grown and maintained according to methods outlined in the Cold Spring Harbor manual (6). Strains were propagated on rich (yeast-peptone-dextrose [YPD]) or synthetic complete (SC) medium, dropping out certain amino acids to select for plasmid retention, and supplemented with 5 mM GdnHCl or 5-fluoroorotic acid (5-FOA) where indicated. For the raffinose growth phenotype, glucose was replaced with raffinose and the medium was supplemented with 1 μ g/ml antimycin (Sigma). Plates were grown at 30°C for 3 days unless otherwise noted.

The BY4741 $[SWT^+]$ $[PIN^+]$, BY4741 *swi1* Δ , and c10B-49 $[swi^-]$ $[pin^-]$ *MAT* α strains were described previously (14, 20). The BY4741 $[SWT^+]$ $[pin^-]$ strain was generated by transforming BY4741 $[swi^-]$ $[pin^-]$ cells with cell extract prepared from 74D-694 $[SWT^+]$ $[pin^-]$ *TRP1::TEF1-RNQ1CFP* using a procedure similar to what was described previously (14). To create 74D-694 $[SWT^+]$ $[pin^-]$ *TRP1::TEF1-RNQ1CFP*, 74D-694 $[SWT^+]$ $[pin^-]$ cells were transformed with a HindIII-linearized p304TEF-RNQ1CFP to integrate the *TEF1-RNQ1CFP* cassette into the *TRP1* locus (*RNQ1CFP* was used as a marker for monitoring the prion status of Rnq1).

To construct the BY4741 *swi1* Δ $[SWT^+]$ /p416TEF-SWI1 strain, the $[SWT^+]$ prion was introduced to BY4741 *swi1* Δ by extract transformation from a c10B $[SWT^+]$ *kar1-1* strain generated in our laboratory (14, 15). Log-phase c10B $[SWT^+]$ *kar1-1* cells were suspended in 100 μ l STC buffer (1 M sorbitol, 10 mM Tris-HCl, 10 mM CaCl₂) and vortexed 4 times for 1 min each time in a bead beater (Biospecs) to lyse them. Fifty microliters of the crude lysates was transformed into the BY4741 *swi1* Δ /p416TEF-SWI1 strain using a standard yeast transformation protocol (see below), with p413TEF as a transformation marker. His⁺ Ura⁺ transformants were screened for the presence of $[SWT^+]$ by streaking them onto medium containing raffinose as a sole carbon source. Transformants showing reduced growth on raffinose were then transformed with p415TEF-SWI1₁₋₄₆-YFP. Those isolates that exhibited Swi1₁₋₄₆-yellow fluorescent protein (YFP) aggregation (which forms aggregates in $[SWT^+]$ cells [see Fig. 3A]) were treated with 5 mM GdnHCl to cure the prion. Only isolates showing GdnHCl-curable aggregation and reduced growth on raffinose-containing media were considered $[SWT^+]$. One of these isolates was designated *swi1* Δ $[SWT^+]$ /p416TEF-SWI1 and used for the experiments described here.

Plasmid construction. All constructs expressing truncation mutants were derived from p416TEF-NYFP, which was described previously (14). To construct the amino-terminal truncations, fragments of the *SWI1* gene were amplified from genomic DNA using unique forward primers containing SpeI restriction sites and a common reverse primer containing a BamHI restriction site. Carboxyl-terminal truncations were amplified using a common forward primer containing an SpeI site and unique reverse primers containing BamHI sites. The amplified fragments were digested, purified, and ligated into the SpeI and BamHI sites of p416TEF-NYFP, replacing the Swi1N fragment with the truncated version. The correct insertion and sequence of each fragment were verified by sequencing.

To make p415TEF-SWI1₁₋₃₇-GFP, the sequence encoding green fluorescent protein (GFP) was amplified from pFA6a-kanMX6-P3nmt1-GFP (2) using a forward primer containing a BamHI restriction site and a reverse primer containing an XhoI restriction site. The amplified fragment was digested, purified, and ligated into the BamHI and XhoI sites of p416TEF-SWI1₁₋₃₇-YFP, yielding the plasmid p416TEF-SWI1₁₋₃₇-GFP. The fragment encoding Swi1₁₋₃₇-GFP was cut out of p416TEF-SWI1₁₋₃₇-GFP using SpeI and XhoI and ligated into the same sites in p415TEF (27).

To make p416TEF-SWI1mCherry, the sequence encoding mCherry was am-

plified from pmCherry-C1, a derivative of pEGFP-C1 (Clontech), using forward and reverse primers containing XhoI restriction sites. The amplified fragment was digested, purified, and ligated into the XhoI site of p416TEF-SWI1 (14). The correct orientation of the mCherry fragment was determined by restriction analysis. Finally, the *SWI1* stop codon was mutated by site-directed mutagenesis to fuse in frame with mCherry. The correct insertion and sequence of *mCherry* was confirmed by sequencing.

To create the plasmid p304TEF-RNQ1CFP, a 1-kb fragment containing the *GPD* promoter and the *CYC1* terminator cassette was digested from p426GPD (ATCC) and ligated into pRS304 to give rise to p304GPD-CYC1 through the KpnI/SacI sites. The 1.3-kb *CFP* fragment from pECFP-N1 (Clontech) was used to replace the *GFP* fragment of p316CUP1-RNQ1GFP (26) through SacII/NaeI. The resulting plasmid, p316CUP1-RNQ1CFP, was partially digested with HpaI/BamHI to give rise to a 2.1-kb *RNQ1CFP* fragment, which was ligated into p304GPD-CYC1 that was predigested with HincII/BamHI, yielding p304GPD-RNQ1CFP. Subsequently, the *GPD* promoter of p304GPD-RNQ1CFP was replaced by the *TEF1* promoter from p416TEF-SWI1 (20) through the SacI/SpeI sites, resulting in p304TEF-RNQ1CFP.

Table 1 shows a list of primers used for this study.

Yeast transformation. Yeast cells were transformed according to a protocol adapted from the Cold Spring Harbor manual (6). Briefly, log-phase cells were collected by centrifugation, resuspended in LiPEG transformation solution (0.2 M lithium acetate, 40% polyethylene glycol [PEG] 3500), and supplemented with 2 μ l of single-stranded salmon sperm carrier DNA (10 mg/ml; Sigma). One microliter of plasmid DNA (Qiagen miniprep) was then added, and the transformation mixture was vortexed briefly and incubated at 42°C for 15 to 45 min before being plated to selective medium. The plates were incubated at 30°C for 3 days until colonies appeared.

Microscopy. Microscopy images were taken with a Zeiss Axiovert 200 epifluorescence microscope. Samples were viewed with a 100 $\times$ objective and filters specific for differential interference contrast (DIC), YFP/GFP, or mCherry. Images were captured with an AxioCam HR camera (Zeiss) using Axiovision AC (Zeiss).

Cytoduction. Donor and recipient cells for cytoductions were grown separately in selective media overnight. Three hundred microliters of each overnight culture was then combined with 600 μ l YPD rich medium, and the mixture was incubated at 30°C with agitation for 4 to 6 h. Aliquots of the cytoduction mixture were plated to selective medium and grown at 30°C for 3 days. Individual colonies were picked to selective medium to confirm correct nuclear markers.

Immunoblotting. Samples for immunoblots were prepared by alkaline lysis as follows. One milliliter of an overnight yeast culture was pelleted, resuspended in 100 μ l of 0.1 M NaOH, and incubated at room temperature for 5 min. The cells were then pelleted and resuspended in 100 μ l 2 $\times$ Laemmli sample buffer. The resuspended samples were boiled for 5 min and sonicated for 15 1-s pulses. The lysates were then spun down to pellet debris, and 5 μ l of the supernatants was immediately loaded on a 7.5% (for larger peptides) or 15% (for smaller peptides) SDS-PAGE gel. The gels were transferred to polyvinylidene difluoride (PVDF) membranes and blotted with 1:2,500 mouse anti-GFP primary antibody (JL-8; Clontech) and 1:2,500 horseradish peroxidase (HRP)-conjugated rabbit anti-mouse secondary antibody (Thermo Scientific). The resulting chemiluminescence was detected using ECL Western Blotting Detection Reagents (Amersham).

Reverse transcription-PCR. Total RNA was isolated from overnight cultures using the RNeasy Mini kit (Qiagen). cDNA was synthesized using the SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Twenty nanograms of cDNA was used as the template for PCR amplification with primers specific for the Swi1 C terminus (Swi1 RT fwd, 5'-TCTTCGCGCCAGAGTTAGG-3', and Swi1 RT rev, 5'-CCGAGTATTGC CAAGGAGTC-3'). The PCR products were run on a 2% agarose gel with the GeneRuler 100-bp Plus DNA ladder (Fermentas) and imaged on a Bio-Rad gel imager (Bio-Rad).

RESULTS

Swi1₁₋₃₇ decorates prion aggregates in $[SWT^+]$ cells. We previously showed that the carboxyl-terminal portion of Swi1 is sufficient for readily assayable Swi1 functions, since it complements the carbon source usage defect of genomic *SWI1* deletion (15). We also showed that *in vitro*-formed Swi1₁₋₃₂₇ fibrils can induce *de novo* $[SWT^+]$ and that chromosomal deletion of the coding region for Swi1₁₋₃₂₇ results in $[SWT^+]$ loss (14).

TABLE 1. Primers used in this study

Primer name	Primer sequence ^a	Resulting plasmid
Swi1N rev	5'-TGGATCCCGGGCAGAGAACA-3'	Amino-terminal truncations
Swi1N fwd	5'-ATCTAGA <u>ACTAGT</u> ATGGTTTCTTTAATTT-3'	Carboxy-terminal truncations
55 fwd	5'-TACTACTAGTATGAACAATACTAATAACAACAATACGAATACC-3'	p416TEF-SWI1 ₅₅₋₃₂₇ -YFP
100 fwd	5'-TACTACTAGTATGAATAACAGTAACACGGTAGCCTCC-3'	p416TEF-SWI1 ₁₀₀₋₃₂₇ -YFP
156 fwd	5'-TACTACTAGTATGAACAGCCTGTCTCTCAGGC-3'	p416TEF-SWI1 ₁₅₆₋₃₂₇ -YFP
206 fwd	5'-TACTACTAGTATGATCACCAATGTTCAATCCATCAG-3'	p416TEF-SWI1 ₂₀₆₋₃₂₇ -YFP
250 fwd	5'-TACTACTAGTATGTCTAACCAGCTTATTTCTAATTACGC-3'	p416TEF-SWI1 ₂₅₀₋₃₂₇ -YFP
225 rev	5'-GGTGGATCCTTTGTGTTTCGG-3'	p416TEF-SWI1 ₁₋₂₂₅ -YFP
176 rev	5'-GGTGGATCCAAATTGGAAGAATC-3'	p416TEF-SWI1 ₁₋₁₇₆ -YFP
134 rev	5'-GGTGGATCCTTAGCAGCTTTTC-3'	p416TEF-SWI1 ₁₋₁₃₄ -YFP
74 rev	5'-GGTGGATCCTGAAAATCGTCTAC-3'	p416TEF-SWI1 ₁₋₇₄ -YFP
65 rev	5'-GGTGGATCCTTGGTATTCGTATTGTTGTTATTAGTATTG-3'	p416TEF-SWI1 ₁₋₆₅ -YFP
46 rev	5'-GTTGGATCCCTATTGTTGTTATTGTTATTAGCAGG-3'	p416TEF-SWI1 ₁₋₄₆ -YFP
41 rev	5'-GTTGGATCCGTATTATTAGCAGGATTATTATTATT-3'	p416TEF-SWI1 ₁₋₄₁ -YFP
37 rev	5'-GTTGGATCCGGATTATTATTATTAGTATTATTATT-3'	p416TEF-SWI1 ₁₋₃₇ -YFP
31 rev	5'-GTTGGATCCGTATTATTATTATTAGTATTATTGTTATT-3'	p416TEF-SWI1 ₁₋₃₁ -YFP
GFP fwd	5'-TATGGATCCATCTTTAATTAACAGTAAAGGAGAAGAAC-3'	p416TEF-SWI1 ₁₋₃₇ -GFP
GFP rev	5'-ATACTCGAGCTATTGTATAGTTTCATCCATGCCATG-3'	p416TEF-SWI1 ₁₋₃₇ -GFP
mCherry fwd	5'-TATCTCGAGGTGAGCAAGGGCGAGGAG-3'	p416TEF-SWI1mCherry
mCherry rev	5'-TATCTCGAGCTACTTGTACAGCTCGTCCATG-3'	p416TEF-SWI1mCherry

^a The underlined nucleotides indicate restriction sites used for cloning.

Furthermore, Alberti et al. demonstrated that a peptide comprising a considerable portion of the Q/N-rich Swi1 N terminus (Swi1₁₋₃₈₅) forms SDS-resistant aggregates *in vivo* and amyloid fibers *in vitro* (1). Thus, we hypothesized that the minimal Swi1 PrD was located within the first 327 amino acids of Swi1 (here designated Swi1N). We reasoned that a domain that could propagate [SWT⁺] would also aggregate in [SWT⁺] cells; thus, we first aimed to identify the minimal region of Swi1N that could decorate [SWT⁺] aggregates.

To address this question, we constructed a series of Swi1N

truncations fused to a C-terminal YFP reporter (Fig. 1). These fusion proteins were then expressed in isogenic [swi⁻] [PIN⁺] and [SWT⁺] [PIN⁺] cells from a constitutive *TEF1* promoter. All fusion proteins were expressed at the expected molecular weights in both [swi⁻] and [SWT⁺] cells (Fig. 2B and 3B). The Swi1N-YFP control and all of the truncation fusion proteins exhibited diffuse fluorescence in [swi⁻] cells (Fig. 2A and 3A), indicating that no aggregation occurred as a result of overexpression. The positive control, Swi1N-YFP, formed fluorescent puncta in [SWT⁺] cells, as expected (Fig. 2A). Deletion of 55 or more amino acids from the Swi1N amino terminus, however, resulted in loss of aggregation: all fusion proteins representing Swi1N amino-terminal truncation exhibited diffuse fluorescence in [SWT⁺] cells (Fig. 2A). This suggests that the extreme amino terminus of the protein is important for aggregation. Indeed, truncation of Swi1N from the carboxyl terminus revealed that only the extreme amino terminus is required for aggregation, as a fusion protein representing only the first 37 amino acids of Swi1 exhibited robust aggregation in [SWT⁺] cells (Fig. 3A). Further truncation to 31 amino acids resulted in complete loss of aggregation (Fig. 3A). Taken together, these data indicate that the extreme amino terminus of Swi1, a region of 37 amino acids, is sufficient for decoration of Swi1 aggregates in [SWT⁺] cells.

Swi1₁₋₃₇ maintains the prion conformation in the absence of Swi1. Given that Swi1₁₋₃₇ decorates [SWT⁺] aggregates in cells expressing Swi1, we next asked whether Swi1₁₋₃₇ was able to maintain the aggregated prion conformation by itself (in the absence of full-length Swi1). To test this, we needed to construct a [SWT⁺] strain coexpressing Swi1 and Swi1₁₋₃₇ in which Swi1 expression could be “turned off,” since maintenance of a prion requires a continuous supply of soluble prion protein. We started by transforming a *swi1Δ* strain with p416TEF-SWI1. The p416TEF-SWI1 plasmid is maintained by Ura⁺ selection, so it can be counterselected for by treatment with 5-FOA (4). Next, we introduced the [SWT⁺] prion by extract transformation (see Materials and Methods for details), result-

FIG. 1. Truncation mutants used in this study. (A) Schematic of the Swi1 protein and truncation peptides used in the aggregation study (not to scale). Swi1N was previously shown to decorate [SWT⁺] aggregates (14) and is used as a positive control. The numbers indicate amino acids. All truncation mutants are fused with a C-terminal YFP reporter. (B) Amino acid sequence of Swi1₁₋₃₇, the region identified as the minimal Swi1 PrD in this study. Asparagine residues are highlighted in blue.

FIG. 2. Deletion of the extreme amino terminus of Swi1 abolishes aggregation in *[SWT⁺]* cells. (A) Microscopy images ($\times 100$) of *[swi⁻]* and *[SWT⁺]* cells expressing Swi1N amino-terminal truncation mutants fused to YFP. Ten to 20 individual transformants were imaged for each truncation; representative images are shown. The numbers indicate the average percentages of *[SWT⁺]* cells with aggregates (agg). (B) Western blot of the Swi1N amino-terminal-truncation YFP fusions. A polyclonal anti-GFP antibody was used to detect the fusion proteins.

ing in the strain *swi1 Δ [SWT⁺] [pin⁻]/p416TEF-SWI1* (Fig. 4A). This strain was then transformed with p415TEF-SWI1₁₋₃₇GFP (Fig. 4A). As expected, the fusion protein formed fluorescent foci in these cells, due to Swi1₁₋₃₇-GFP decoration of preexisting *[SWT⁺]* aggregates formed from full-length Swi1 (Fig. 4B and C). These cells exhibited an intermediate or slow growth phenotype on raffinose as a sole carbon source (defined as *Raf^{+/-}*), due to the presence of the prion (Fig. 4D, pre-5-FOA). The *swi1 Δ [SWT⁺] [pin⁻]/p416TEF-SWI1/p415TEF-SWI1₁₋₃₇GFP* cells were then treated with 5-FOA to select for cells that had lost the *URA3*-based plasmid p416TEF1-SWI1 (Fig. 4A). After 5-FOA treatment, Swi1₁₋₃₇-GFP still formed fluorescent foci (Fig. 4B and C), suggesting that it is able to maintain the *[SWT⁺]* conformation in the absence of full-length Swi1. To confirm that these cells were in fact cured of the p416TEF-SWI1 plasmid, they were spotted to medium containing raffinose as the sole source of carbon before and after treatment with 5-FOA. If p416TEF-SWI1 was successfully eliminated by 5-FOA treatment, then the lack of Swi1 should result in no growth at all on raffinose-containing medium (*Raf⁻*). Thus, 5-FOA treatment should result in a transition from a *Raf^{+/-}* phenotype to a *Raf⁻* phenotype. This is indeed what we saw: before 5-FOA treatment, the *swi1 Δ [SWT⁺] [pin⁻]/p415TEF-SWI1₁₋₃₇GFP/p416TEF-SWI1* strain was *Raf^{+/-}*, and after 5-FOA treatment, the resulting *swi1 Δ [SWT⁺] [pin⁻]/p415TEF-SWI1₁₋₃₇GFP* strain was *Raf⁻* (Fig. 4D). The absence of *SWI1* expression was also confirmed by

reverse transcription-PCR (data not shown). Thus, we conclude that Swi1₁₋₃₇ maintains the *[SWT⁺]* prion fold on its own in the absence of full-length Swi1 protein.

Swi1₁₋₃₇ can transmit the *[SWT⁺]* prion conformation to soluble Swi1. Since Swi1₁₋₃₇ is able to maintain the *[SWT⁺]* aggregation phenotype by itself, we next asked whether the peptide can transmit the prion fold back to full-length Swi1. To test this, the *swi1 Δ [SWT⁺]/p415TEF-SWI1₁₋₃₇GFP* strain generated as described above was transformed with p416TEF-SWI1mCherry (Fig. 5). Using a tagged version of the full-length protein enabled us to test for growth on raffinose as well as aggregation, two important *[SWT⁺]* phenotypes. When imaged for mCherry fluorescence, approximately 95% of *swi1 Δ [SWT⁺]/p415TEF-SWI1₁₋₃₇GFP/p416TEF-SWI1mCherry* cells exhibited a punctate pattern of fluorescence, a percentage comparable to that of the *[SWT⁺]* control strain (Fig. 5B and C). This indicates that Swi1₁₋₃₇ alone can induce aggregation of soluble, full-length Swi1 protein. These cells were then spotted to medium containing raffinose as the sole carbon source. The *swi1 Δ [SWT⁺]/p415TEF-SWI1₁₋₃₇GFP/p416TEF-SWI1mCherry* strain showed a *Raf^{+/-}* phenotype, indicating that Swi1₁₋₃₇ can in fact transmit the *[SWT⁺]* phenotype to full-length Swi1 (Fig. 5D). Finally, the *swi1 Δ [SWT⁺]/p415TEF-SWI1₁₋₃₇GFP/p416TEF-SWI1mCherry* strain was passaged twice on medium containing 5 mM GdnHCl, which cures the *[SWT⁺]* prion (15). After GdnHCl treatment, growth of these cells on raffinose was restored to levels comparable to that of *[swi⁻]* cells (Fig. 5D),

FIG. 3. The extreme amino-terminus of Swi1 is sufficient for aggregation in *[SWT⁺]* cells. (A) Microscopy images ($\times 100$) of *[swi⁻]* and *[SWT⁺]* cells expressing Swi1N carboxyl-terminal truncation mutants fused to YFP. Ten to 20 individual transformants were imaged for each truncation; representative images are shown. The numbers indicate average percentages of *[SWT⁺]* cells with aggregates (agg). (B) Western blot of the Swi1N carboxyl-terminal-truncation YFP fusions. A polyclonal anti-GFP antibody was used to detect the fusion proteins.

indicating that the *Raf^{+/−}* phenotype was based on a prion-like phenomenon.

Swi1₁₋₃₇ can transmit *[SWT⁺]* to endogenous Swi1. The results described above demonstrate that aggregated Swi1₁₋₃₇ can propagate the *[SWT⁺]* prion conformation within a carefully designed system. We next asked whether Swi1₁₋₃₇ can also transmit *[SWT⁺]* to soluble Swi1 in a naïve strain with a wild-type genetic background. To address this question, we cytoduced the *swi1Δ [SWT⁺]/p415TEF-SWI1₁₋₃₇-GFP* strain generated as described above (donor) to a *[swi⁻]/p416TEF-SWI1mCherry* reporter strain (recipient) (Fig. 6A). The recipient strain carries the *kar1-1* mutation, which prevents nuclear fusion; the resulting “cytoductants” therefore contain either the donor or recipient nucleus and the cytoplasmic contents of both donor and recipient cells. Seven cytoductants containing only the recipient nucleus were tested for Swi1mCherry aggregation and reduced growth on raffinose-containing media. When these cells were imaged for mCherry fluorescence, five out of seven cytoductants showed Swi1mCherry aggregation (one representative cytoductant with aggregation is shown in Fig. 6B). The expression of the Swi1mCherry fusion protein is low, which may account for the lack of visible aggregates in two of the seven cytoductants. We previously showed that the expression

of exogenous Swi1 is tightly controlled by the cell, resulting in very low expression levels, even from strong constitutive promoters (14). All seven cytoductants showed reduced growth on raffinose (*Raf^{+/−}*), indicating transmission of the *[SWT⁺]* conformation to Swi1mCherry (one representative cytoductant is shown in Fig. 6C). Thus, we conclude that Swi1₁₋₃₇ aggregates can transmit *[SWT⁺]* conformation to wild-type cells expressing endogenous Swi1 by cytoplasmic mixing.

DISCUSSION

The PrD of a yeast prion protein is the minimal region required for propagation and transmission of the prion. In this study, we have demonstrated that the first 37 amino acids of Swi1 represent the minimal PrD of the protein. Swi1₁₋₃₇ forms prion-like aggregates in the cytoplasm of *[SWT⁺]* cells, but not *[swi⁻]* cells, indicating that it can decorate preexisting *[SWT⁺]* aggregates (Fig. 3A). The peptide remains aggregated in the absence of full-length Swi1, indicating that by itself it is sufficient for propagation of the prion fold (Fig. 4). Aggregated Swi1₁₋₃₇ is also able to induce full-length Swi1 to adopt the prion conformation, thus demonstrating that it is capable of transmitting *[SWT⁺]* (Fig. 5). Finally, Swi1₁₋₃₇ can transmit the

FIG. 4. Swi1₁₋₃₇ maintains the [SWT⁺] phenotype in the absence of Swi1. (A) Schematic of the experimental design. A *swi1Δ* [SWT⁺]/p416TEF-SWI1 strain was transformed with p415TEF-SWI1₁₋₃₇GFP. The cells were treated with 5-FOA to select for those that had lost p416TEF-SWI1. (B) Microscopy images (×100) of cells before and after treatment with 5-FOA. Ten colonies were imaged for both the pre-5-FOA and post-5-FOA groups; representative images are shown. (C) Quantification of cells with aggregates. Fifty individual cells were counted from one representative colony. The error bars indicate standard deviations. (D) Loss of [SWT⁺] was confirmed by the growth phenotype on medium containing raffinose as the sole carbon source. Log-phase cells were serially diluted 1:10 and spotted to rich medium (YPD) or raffinose medium (raffinose). Ten colonies were tested; representative images are shown. Wild-type (wt), [SWT⁺], and *swi1Δ* controls are included.

[SWT⁺] conformation to endogenous Swi1 by cytoplasmic mixing (Fig. 6). Due to the lack of a positive selection system for [SWT⁺] cells, we were unable to test whether Swi1₁₋₃₇ is sufficient for *de novo* induction of [SWT⁺] in naïve cells. While Swi1₁₋₃₁ did not form visible aggregates in [SWT⁺] cells, we cannot exclude the possibility that smaller oligomeric forms could propagate the prion.

The minimal Swi1 PrD defined here is unique among known PrDs in that it contains no glutamine residues (Fig. 1B). The yeast PrDs defined to date are highly enriched in both glutamine and asparagine, and screens used to detect new prion candidates have been based on parameters considering both amino acids to be present in large quantities (1, 26). Our results show that an asparagine-rich, glutamine-free PrD is capable of propagating and transmitting a yeast prion. Inter-

estingly, Swi1₁₋₃₇ also lacks glycine residues (which are biased for in mammalian PrP and the PrDs of Sup35 and Rnq1 [18]), as well as tyrosine and serine residues (which are mildly biased for in some yeast prion PrDs [18]). In a recent genome-wide screen for yeast prion protein candidates, the authors noted that the asparagine-rich candidates they identified were more likely to be amyloidogenic than glutamine-rich candidates (1). Our results correlate well with this observation. While one study did show that a polyasparagine peptide [poly(N104)] could form SDS-insoluble, Hsp104-dependent aggregates in yeast, this peptide does not demonstrate the heritability that is characteristic of prions and, at 104 amino acids, was much larger than the asparagine-rich PrD described here (35).

Another notable feature of the Swi1 PrD is its highly homogeneous amino acid composition: 60% of the residues com-

FIG. 5. Swi1₁₋₃₇ can transmit [SWT⁺] to full-length Swi1. (A) Schematic of the experimental design. A *swi1Δ* [SWT⁺]/pSWI1₁₋₃₇GFP strain was transformed with p416TEF-SWI1-mCherry. (B) Microscopy images (×100) of cells expressing Swi1mCherry. [swi⁻] and [SWT⁺] control cells are included. Ten transformants each from 3 individual transformations were imaged for mCherry aggregation (*swi1Δ* [SWT⁺]/pSWI1₁₋₃₇GFP); five transformants from three individual transformations were imaged for [swi⁻] and [SWT⁺] control strains. Representative images are shown. (C) Quantification of transformants with aggregates. One hundred cells from three individual transformants were counted for each group. The error bars indicate standard deviations. (D) One transformant from each of three individual transformations was tested for reduced growth on raffinose-containing medium (-ura+raff). These same cells were also passaged twice on selective medium containing 5 mM GdnHCl and then restreaked to raffinose-containing (-ura+raff) and dropout (-ura) media. The images shown are representative of all three colonies tested.

prising this domain are asparagine (N) and 30% are threonine (T), with non-N or -T residues representing only 10% of the total composition, making the Swi1 PrD unique in its pronounced enrichment for a single residue. While a role for threonine in prion formation has not been previously described, one study did note enrichment for T residues in the Ure2 PrD (50). This observation, and the high prevalence of T residues in the Swi1 PrD, suggests that T may also play a role in prion formation and propagation. Interestingly, the Ure2 PrD has a greater content of N versus Q than the well-defined Sup35 and Rnq1 PrDs (10). This raises the intriguing possibility that Swi1 and Ure2 may represent a subclass of prion proteins with specialized requirements for PrD composition. We anticipate that the identification and characterization of novel prion proteins in the future will shed light on this hypothesis.

One of the most surprising findings from this study is the strikingly small size of the Swi1 PrD. At a mere 37 amino acids, this domain is smaller than any prion domain defined to date: Sup35₁₋₆₁ can propagate [PSI⁺] *in vitro* (7, 21), although up to 83 amino acids are required for propagation of weaker [PSI⁺] variants (46); the Ure2 PrD was defined as amino acids 1 to 65

(25), and the smallest defined region of Rnq1 required for [PIN⁺] decoration and transmission is even larger (55). However, to our knowledge, no genetic evidence has been reported showing that any of the above-mentioned PrDs are able to propagate their corresponding prions *in vivo*. This means that Swi1₁₋₃₇ is the smallest domain defined to date that is capable of propagating and transmitting a yeast prion *in vivo*. A recent study by Fiumara et al. identified a marked enrichment for coiled-coil motifs in prion proteins and their interactors (16). The authors suggested that a coiled-coil structure may explain the pathogenic threshold for poly(Q) length in the Huntington protein (Htt): a pathogenic expansion region of 36 or more Q residues contains five heptads (a repeat typical of coiled-coil domains), and greater expansion results in an increased tendency to polymerize. Since the Swi1 PrD aggregation threshold is virtually the same length, it is tempting to speculate that coiled-coil structure may also play a role in [SWT⁺] formation. If this is true, then further truncation to 31 amino acids would eliminate the fifth heptad, which could account for its inability to propagate the prion conformation. However, prediction software (Coils and Paricoil2) indicates a very low probability

FIG. 6. Swi1₁₋₃₇ can transmit [SWT⁺] to endogenous Swi1. (A) Schematic of experimental design. A *swi1Δ* [SWT⁺]/p415TEF-SWI1₁₋₃₇GFP strain (donor) was cytoducted to a [swi⁻] *kar1-1*/p416TEF-SWI1-mCherry strain (recipient). Cytoductants were selected for on media lacking uracil and leucine. Authentic cytoductants were selected based on the presence of the recipient cell nuclear marker (Met⁺) and the absence of the donor cell nuclear marker (Lys⁺). (B) Microscopy images (×100) of cytoductant, as well as [swi⁻] and [SWT⁺] control strains containing p416TEF-SWI1-mCherry. Seven cytoductants were imaged; a representative image of a cytoductant exhibiting Swi1-mCherry aggregation is shown. (C) Log-phase cells were serially diluted 1:10 and spotted to YPD and raffinose media. Seven cytoductants were tested; representative results are shown.

of coiled-coil structure within this region (E. T. Crow, unpublished data), and the amino acid sequence does not exhibit the heptad repeat motif characteristic of coiled-coil regions (Fig. 1B). Indeed, all the regions of Swi1 that have a high probability of forming coiled coils are downstream of Swi1₁₋₃₂₇, a domain that we have already shown to be crucial for [SWT⁺] propagation (14). If coiled-coil structure does not in fact play a role in [SWT⁺] aggregation, then the fact that removal of six additional residues from the C terminus of Swi1₁₋₃₇ renders the peptide unable to decorate [SWT⁺] aggregates suggests that these residues might be critical for interactions between monomeric and aggregated Swi1. It is possible that these residues are localized within the core region of [SWT⁺] amyloids. Further structural studies will shed light on the importance of individual residues within Swi1₁₋₃₇ in prionogenesis.

The location of the Swi1 PrD is also surprising: since up to 536 amino acids can be deleted from the amino terminus of the protein without significantly affecting readily assayable Swi1 functions (15), this suggests that the intervening region between the prion domain and the carboxyl terminus may be largely without function. One study demonstrated that the second “quarter” of Swi1 (amino acids 329 to 657) facilitates SWI/SNF interaction with transcriptional activators but also showed that this activity is largely redundant with that of a region of Snf5, another member of the SWI/SNF complex (37), indicating that the middle region of Swi1 is nonessential. A recent paper by Kadnar et al. suggests that multiple prion determinants may exist within a yeast prion domain (20). Our deletion analysis clearly demonstrates that the extreme N terminus of Swi1 is essential for aggregation (since deletion of the first 55 amino acids completely abolishes decoration of [SWT⁺] aggregates); however, we cannot rule out the possibility that

downstream regions may have a modifying effect on [SWT⁺] formation and propagation.

In conclusion, we have shown here that the minimal prion domain of Swi1 consists of the 37 amino acids at the extreme amino terminus of the protein. This domain is unusual in both its size and composition and as such has significant implications for understanding the nature of prion aggregates and for the identification of novel prion proteins.

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