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The *Candida albicans* Sup35p protein (CaSup35p): function, prion-like behaviour and an associated polyglutamine length polymorphism

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The Sup35p protein of *Saccharomyces cerevisiae* is an essential translation factor whose prion-like properties give rise to the non-Mendelian genetic element [*PSI*$^+$]. In this study the *SUP35* gene from the related yeast species *Candida albicans* has been characterized. The *CaSUP35* gene encodes a protein (CaSup35p) of 729 aa which shows 65% amino acid identity to the *S. cerevisiae* Sup35p protein (ScSup35p), with the C-terminal region showing greater identity (79%) than the N-terminal region. The full-length CaSup35p can functionally replace ScSup35p in *S. cerevisiae* although complementation is only complete when CaSup35p is overexpressed. Complementation only requires expression of the CaSup35p C domain. In *S. cerevisiae* the full-length CaSup35p is unable to establish a prion-like aggregated state even in the presence of endogenous ScSup35p prion 'seeds', thus confirming the existence of a species barrier in fungal prion propagation. Subcellular localization studies in *C. albicans* show that although CaSup35p is normally ribosome-associated, when not ribosome-associated, it does not form pelletable high-molecular-mass aggregates characteristic of the ScSup35p in [*PSI*$^+$] strains. Unlike the ScSup35p, the CaSup35p N domain contains a number of polyglutamine repeats although it does contain seven copies of the peptide GGYQQ that is repeated in the ScSup35p N domain. Analysis of the *CaSUP35* gene from 14 different strains of *C. albicans* identified four naturally occurring polymorphisms associated with changes in the length of the largest of the polyglutamine repeats. These findings have important implications for the evolution of fungal prion genes.

Keywords: *SUP35* gene, polyQ, translation termination, [*PSI*$^+$] determinant

INTRODUCTION

Prions are a remarkable class of proteins that were originally identified as the protein-only infectious agents associated with a range of debilitating neurodegenerative disorders of mammals, the transmissible spongiform encephalopathies otherwise known as prion diseases (Prusiner, 1982). More recently, studies with the yeast *Saccharomyces cerevisiae* have identified several prion proteins that are able to act as stable, transmissible determinants of phenotype. Of these yeast prion proteins, two in particular have been intensively studied: Ure2p, which gives rise to the [*URE3*] determinant, and Sup35p, which gives rise to the [*PSI*$^+$] determinant (reviewed by Wickner & Chernoff, 1999).

The *SUP35* gene was originally identified in an *S. cerevisiae* screen for recessive mutations which impaired the maintenance of translation fidelity (Hawthorne & Leupold, 1974). The encoded Sup35p protein was subsequently shown to be a subunit of the eukaryotic release factor forming a heterodimer with a second subunit, Sup45p (also called eRF1) that functions in

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translation termination at all three stop codons (Stansfield *et al.*, 1995b; Zhouravleva *et al.*, 1995). Wickner (1994) suggested that Sup35p, an essential component of the translation machinery, might be a prion protein responsible for the translation-termination-related [*PSI*$^+$] phenotype of *S. cerevisiae*. This hypothesis has now been confirmed by a wide range of biochemical and genetical studies (reviewed by Wickner & Chernoff, 1999).

[*PSI*$^+$] strains have an elevated level of suppressor tRNA-mediated readthrough of nonsense codons compared with wild-type [*psi*$^-$] strains (Cox, 1965; Firoozan *et al.*, 1991). Analysis of a mutation located in the *SUP35* gene (Doel *et al.*, 1994), coupled with the demonstration that the deletion of the non-essential N-terminus of the protein (Ter-Avanesyan *et al.*, 1994) eliminated both the [*PSI*$^+$] phenotype and the increased *de novo* appearance of [*PSI*$^+$] following *SUP35* overexpression (Chernoff *et al.*, 1993), showed that *SUP35* is the [*PSI*] determinant. In [*PSI*$^+$] strains, the majority of the cellular pool of Sup35p, not associated with Sup45p and/or the ribosome, is present as part of a high-molecular-mass aggregate which is incompatible with its translation release factor activity, leading to the translation-termination-associated phenotype characteristic of [*PSI*$^+$] mutants (Patino *et al.*, 1996; Paushkin *et al.*, 1996, 1997). The conversion of soluble Sup35p to the aggregated prion form is promoted by a yet-to-be fully defined conformational change in Sup35p and is driven by the accumulating altered conformer (Serio *et al.*, 2000; Sparrer *et al.*, 2000). Such a self-catalysed propagation of an altered protein conformer parallels the behaviour of the mammalian prion protein PrP (reviewed by Prusiner *et al.*, 1998).

Analysis of the known eukaryotic Sup35p protein sequences suggests they all contain two distinct regions: an evolutionarily conserved C-terminus, which in *S. cerevisiae* is essential for translation termination and hence cell viability, and a less conserved N-terminal extension of varying length and amino acid composition (Kushnirov *et al.*, 1988; Wilson & Culbertson, 1988). The prion conversion of Sup35p is promoted by this N-terminal prion-forming domain (PrD) of Sup35p (reviewed by Tuite, 2000), a region of the protein molecule which is not required for eRF3 activity (Ter-Avanesyan *et al.*, 1993). Within the Sup35p PrD lie a series of five nonapeptide repeats with the core motif GGYQQ. Deletion of the repeat-containing region leads to an inability of the otherwise functional Sup35p to be propagated in its prion-like form (Ter-Avanesyan *et al.*, 1993) and we have recently shown that deletion of even one of the nonapeptide repeats leads to loss of the prion-like behaviour of Sup35p (Parham *et al.*, 2001). Intriguingly the human prion protein PrP also contains a series of sequence-related octapeptide repeats near its N-terminus which have also been implicated in mammalian prion propagation (Flechsig *et al.*, 2000).

Studies on Sup35p have recently been extended to a variety of other fungal species, including *Candida albicans* and *Pichia methanolica* (Santoso *et al.*, 2000; Kushnirov *et al.*, 2000; Chernoff *et al.*, 2000). These studies have indicated that the N-terminal regions of these Sup35p homologues have diverged considerably from the *S. cerevisiae* Sup35p PrD at the amino acid sequence level although they are still Gln/Asn rich. Interestingly, the CaSup35p NM sequence reported by Santoso *et al.* (2000) also contained a number of short poly(Gln) sequences. Replacing the ScSup35p N or NM region with the corresponding heterologous Sup35p region did not prevent the chimaeric Sup35p from forming a prion-like aggregate in *S. cerevisiae*, although such aggregates could only be induced by the overexpression of the corresponding heterologous Sup35p sequence. That overexpression of the ScSup35p NM region did not induce the formation of the chimaeric Sup35p aggregates was taken as evidence for a 'barrier' to interspecies prion propagation in yeasts. Furthermore, Santoso *et al.* (2000) showed that replacing a region of the CaSup35p N domain with residues 8–26 from the ScSup35p N domain overcame this barrier to seeding by ScSup35p NM. Further studies by Chien & Weissman (2001) have indicated that this 'species barrier' is probably due to the different Sup35p N domains forming distinct, non-interacting conformations with the *S. cerevisiae* protein. While the aggregation of chimaeric Sup35p protein can be seeded by homologous N domains in *S. cerevisiae*, there is no evidence that the full-length heterologous Sup35p behaves in the same way in *S. cerevisiae* or is able to establish a prion-like state in its natural host.

To further address the question of the conservation of function and prion-like properties of Sup35p in fungal species other than *S. cerevisiae*, we have isolated and characterized the full-length *SUP35* gene from *C. albicans* (*CaSUP35*). This species of yeast, although closely related to *S. cerevisiae*, is pathogenic in man and is frequently encountered as a commensal of the human digestive system and vaginal tract (Smith, 1989). It has thus evolved to colonize distinct ecological niches to those of *S. cerevisiae*.

METHODS

Bacterial and yeast strains used. *Escherichia coli* strains used were DH5α [*supE44 lacU169* (Δ*lacZ* M15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA*] (Hanahan, 1983) and JM109 [*recA1 supE44 endA1 hsdS17 gyrA96 relA thi* Δ(*lac–proAB*)] (Yanisch-Perron *et al.*, 1985).

Genotypes of the *S. cerevisiae* strains used in this work and their origins are shown in Table 1. The diploid strain MT26 was obtained by mating the two haploid strains BSC783/4a and BSC783/4c. MT26.4 is a derivative of MT26 in which one of the *SUP35* alleles was deleted and replaced with a *kanMX4* allele, as described below. The haploid strains MT26.4/3c and MT26.4/5c were recovered from the meiotic progeny of MT26.4 transformed with pDR4, a centromeric plasmid containing the *URA3* and the *C. albicans SUP35* gene (see below and Table 3). The haploid strain MT700/9d [*PSI*$^+$] was recovered from the meiotic progeny of the diploid strain MT700 transformed with pYK810, a centromeric plasmid

Table 1. Genotypes of strains of *S. cerevisiae* used in this study

All strains were constructed in this laboratory.

Strain	Genotype
BSC483/1a	*MATα SUQ5 ade2-1 his5-2 lys1-1 can1-100 PNM2 ura3-1 sal3* [*psi*$^-$]
JDS3-15	*MAT***a** *SUQ5 ade2-1 his5-2 lys1-1 can1-100 PNM2 ura3-1 sal3* [*psi*$^-$]
PF1-28	*MAT***a** *SUQ5 ade2-1 his5-2 lys1-1 can1-100 PNM2 ura3-1 leu2-3/112* [*psi*$^-$]
BSC783/4a	*MATα SUQ5 ade2-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112* [*psi*$^-$]
BSC783/4c	*MAT***a** *SUQ5 ade2-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112* [*psi*$^-$]
BSC783/4a *sal3-4*	*MATα SUQ5 ade2-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 sal3-4* [*psi*$^-$]
MT26	*MATα/MAT***a** *SUQ5/SUQ5 ade2-1/ade2-1 his3-11/his3-11 his3-15/his3-15 ura3-1/ura3-1 leu2-3/leu2-3 leu2-112/leu2-112* [*psi*$^-$]
MT26.4	As for MT26 but also *sup35::kanMX4/SUP35*$^+$
MT26.4/3c	*MATα sup35::kanMX4 SUQ5 ade2-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112* [*psi*$^-$]
MT26.4/5c	*MAT***a** *sup35::kanMX4 SUQ5 ade2-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112* [*psi*$^-$]
BSC772/18c	*MAT***a** *SUQ5 ade2-1 his4-166 ura3-1 leu2-2 lys1-1 can1-100*
MT700/9d [*PSI*$^+$]	*MATα SUQ5 ade2-1 his3 ura3-1 leu2 sup35::kanMX*
MT700/9d [*psi*$^-$]	*MATα SUQ5 his4 lys1 ura3-1 leu2 sup35::kanMX*

Table 2. Strains of *C. albicans*

Strain	Origin/source
2005E	GlaxoWellcome, UK
10261	ATCC
W01	D. Soll, University of Iowa, USA
IGC 2427	Tree leaves, Gulbenkian Institute, Portugal
WHH1, 3, 4, 7	Clinical samples, J. Reuther, William Harvey Hospital, UK
5*	Skin (Spain)
78/011*	Oral cavity (UK)
81/175*	Fingernail (USA)
81/191*	Blood (USA)
81/192*	Vagina (USA)
J930510*	Oral cavity, AIDS patient (Germany)

* Provided by F. C. Odds (Aberdeen, UK).

containing the *URA3* and the *S. cerevisiae SUP35* genes (Kikuchi *et al.*, 1988; Table 3). The MT700/9d [*psi*$^-$] derivative was created by growth in the presence of 3 mM GuHCl (Tuite *et al.*, 1981). The diploid strain MT700 was obtained by crossing MT26.4/3c with BSC772/18c. Table 2 provides details of all laboratory and clinical strains of *C. albicans* used in this study.

Yeast growth conditions. Standard yeast media, cultivation procedures and genetic techniques were used (Kaiser *et al.*, 1994). Tetrad dissection was performed on a Singer Micromanipulator (model MSM System). Yeast cultures were grown at 30 °C. The presence of the [*PSI*] determinant was monitored phenotypically by suppression of the ochre mutation *ade2-1* in strains carrying the ochre suppressor tRNA^{Ser} gene *SUQ5*. This allows for a direct visualization of allosuppression by colony-colour and/or adenine prototrophy (Cox, 1965). Thus [*psi*$^-$] strains are red on complete (YPD) medium and do not grow on medium lacking adenine (SD − Ade) whereas [*PSI*$^+$] strains are white or pink on YPD medium and grow on SD − Ade after 4 days incubation at 30 °C. To enhance the red colony-colour, strains were grown on 1/4YPD solid medium (4%, w/v, glucose; 1%, w/v, Bactopeptone; 0·25%, w/v, yeast extract; 2%, w/v, agar). Elimination of [*PSI*$^+$] with 3 mM GuHCl (Tuite *et al.*, 1981) was performed as previously described. Resistance to canavanine was monitored in defined minimal media supplemented with canavanine (25 μg ml^{-1}). Geneticin (G418)-resistant strains were selected in YPD + G418 (200 μg ml^{-1}).

Recombinant DNA techniques. Standard protocols were used for nucleic acid isolation, electrophoresis, fragment purification, restriction enzyme digestion, Southern blotting and PCR (Sambrook *et al.*, 1989). Restriction enzymes and *Taq* DNA polymerase (Expand) were purchased from Boehringer Mannheim. For Southern hybridization analysis of isolated plasmid DNA, DNA probes were labelled with DIG-11-dUTP (Boehringer Mannheim) using the random primer method (Feinberg & Vogelstein, 1983) and following the manufacturer's instructions. Hybridization was carried out using a nylon membrane (Hybond-N; Amersham) according to the manufacturer's instructions, as was luminescent detection (Boehringer Mannheim) of bound probe. For Southern blot analysis of *C. albicans* genomic DNA, a *CaSUP35* gene probe

Table 3. Plasmids used in this study

Plasmid	Construct	Reference
pDR1	*CaSUP35* homologue in YCp50	This study
pDR4	*CaSUP35* gene in pRS416	This study
pUKC606	*ScSUP35* in pRS425	Stansfield *et al.* (1995b)
pYK810	*ScSUP35* in YCp50	Kikuchi *et al.* (1988)
pUKC1508	*ScSUP35* promoter in pRS313	This study
pMA3a	*LEU2*d-2μ ARS	Spalding & Tuite (1989)
pDR6	*CaSUP35* gene in pMA3a	This study
pUKC1530	*CaSUP35* gene in pRS313	This study
pUKC1530-C	*CaSUP35* C domain in pUKC1508	This study
pUKC815-L	*LEU2*, *CEN*, *PGK1–lacZ* fusion	Stansfield *et al.* (1995a)
pUKC817-L	*LEU2*, *CEN*, *PGK1–TAA–lacZ* fusion	Stansfield *et al.* (1995a)

was obtained from the plasmid pDR4 (Table 3) as a 2 kb *Spe*I–*Kpn*I fragment and labelled with [α^{32}P]dCTP by the random primer method (Amersham), according to the manufacturer's instructions.

Isolation and sequencing of the *C. albicans SUP35* gene. Plasmid pDR1 (Table 3) containing the *C. albicans SUP35* gene (*CaSUP35*) was isolated from a *C. albicans* genomic library cloned into the yeast single-copy shuttle YCp50 vector (Rose *et al.*, 1987). The presence of the Sup35p homologue was expected to restore efficient translation termination and this could be monitored phenotypically by antisuppression of the *can1-100* (UAA) mutation in a strain carrying the ochre suppressor tRNase^r gene *SUQ5*. Complementation of a *sal3-4* allosuppressor allele (Crouzet & Tuite, 1987) of the *SUP35* gene in *S. cerevisiae* strain BSC483/1a (Table 1) was performed as follows. After transformation of BSC483/1a by the lithium acetate method of Ito *et al.* (1983), cells were incubated for a further 18 h at 30 °C, with aeration, in defined liquid minimal medium (SD − Ura). Cells were then plated on to SD − Ura + canavanine (25 μg ml^{-1}) to allow for simultaneous selection of transformants that were Ura$^+$ and canavanine resistant, the latter arising from the reduced efficiency of suppression of the *can1-100* allele.

The plasmid pDR1 identified by this strategy further resulted in simultaneous loss of the suppression phenotype for several other ochre mutations in the strain, i.e. *ade2-1*, *his5-2* and *lys1-1*. pDR1 transformants were then assayed for co-loss of the complementing phenotype simultaneously with loss of the pDR1 plasmid-borne *URA3* marker following culturing in non-selective YPD medium. Transformation of a second strain JDS3-15 (Table 1) was used to confirm the complementing phenotype of pDR1.

The cloned *CaSUP35* gene was sequenced by the chain-termination method (Sanger *et al.*, 1977) by either Advanced Biotechnology Centre (Charing Cross & Westminster Medical School, London, UK) or MWG Biotech (Milton Keynes, UK). A variety of subclones derived from pDR1 were generated to facilitate gene localization and DNA sequencing. Both strands of the *CaSUP35* gene were sequenced and the sequence data deposited at GenBank (accession no. AF020554). The N domains of the *CaSUP35* gene were PCR-amplified from 14 different *C. albicans* strains (Table 2), using 'high fidelity' *Pwo* polymerase (Roche) with a 3′ to 5′ proofreading activity, and oligonucleotide primers NTCaSUP35I (ACTGACGGATCCGCTTGGAATGAGGTCTTC) and NTCaSUP35III (CAGGAGCTGGAGCTGGAGAT). Both strands of each of the *CaSUP35* N domains were sequenced and the sequence data deposited with GenBank (accession nos AY028660–AY028673).

Plasmid constructions. Plasmid pDR2 was made by subcloning a 5·2 kb *Bam*HI–*Sal*I fragment from plasmid pDR1 into plasmid pRS416 (Sikorski & Hieter, 1989). This DNA fragment carried a region showing homology to the *S. cerevisiae SUP35* (*ScSUP35*) gene probe as detected by the Southern blot analysis (data not shown). A 1·3 kb *Eco*RV fragment that did not hybridize to the *ScSUP35* gene was deleted from the plasmid pDR2 to produce the *URA3*-based centromeric plasmid pDR4. The multicopy plasmid pDR6, containing the complete *CaSUP35* gene, was constructed by subcloning the 3·9 kb *Bam*HI–*Sal*I fragment from pDR4 into the *LEU2*d-2μ plasmid pMA3a (Spalding & Tuite, 1989). Plasmid pUKC1530 contained the complete *CaSUP35* gene, including the promoter, cloned into pRS313 (Sikorski & Hieter, 1989) as a *Bam*HI–*Sal*I fragment generated from plasmid pDR4.

Plasmid pUKC1530-C, expressing the C domain of CaSup35p, was constructed as follows. The fragment containing the *CaSUP35* C domain was PCR-amplified from plasmid pDR4 using oligonucleotide primers CaSUP35*Spe*I (GAAGAT ACTAGTGACGAAGAAGTTGTCAAG) and CaSUP35*Sac*I (GAGAAAGAGCTCCCGTTGACTCCATTGAGTTC) bearing extensions with *Spe*I and *Sac*I restriction sites respectively (underlined). The suitably digested amplified fragment was then inserted into the plasmid pUKC1508 (with the *SUP35* promoter inserted into pRS313) to generate pUKC1530-C.

Construction of an *S. cerevisiae SUP35* gene deletion strain for plasmid shuffling. The *sup35*::*kanMX4* gene disruption was constructed by a directed PCR-mediated gene deletion strategy (Wach *et al.*, 1994) using the plasmid pFA6a-KanMX4 as template. The PCR primers used were: S1, TGCTCGGAATAACATCTATATCTGCCCACTAGCAACAATGCGTACGCTGCAGGTCGAC; and S2, ATTATTGTGTTTGCATTTACTTATGTTTGCAAGAAATTTAATCGATGAATTCGAGCTCG. Oligonucleotides S1 and S2 were designed such that their 5′ regions were complementary to the sequence flanking the chromosomal *SUP35* coding sequence, while the 3′ portions were complementary to the sequence flanking the *kanMX4* cassette. The resulting PCR-amplified 1·59 kb *kanMX4* cassette, with flanking extensions homologous to the sequences flanking the *ScSUP35* gene, was then transformed into strain MT26 (Table 1) and *sup35*::

kanMX4/SUP35 heterozygous transplacements selected on YPD + G418 (200 μg ml^{-1}) plates and verified by PCR (data not shown). The PCR primers used were: A1, CGCATTAGCTCGCTATTTG; A2, GCTGATTGTTGCCTTGG; A3, GAGAGATCAAGGTACCAC; A4, GATGATGATGATGCCGAG; K2, GATTGCCCGACATTATCGCG; and K3, TATGGAACTGCCTCGGTG. Primers A1–A4 were complementary to the *ScSUP35* sequence while K2 and K3 were complementary to the *kanMX4* cassette and were designed for PCR verification of the expected *sup35*::*kanMX4* replacement. The resulting kanamycin-resistant diploid strain, MT26.4, was then transformed with pDR4 (Table 3), and two haploid spores with the *sup35*::*kanMX4* disruption carrying the pDR4 plasmid (designated MT26.4/3c and MT26.4/5c; Table 1) were identified amongst the meiotic progeny.

Plasmid shuffling to test CaSup35p function. *SUP35* genes were introduced either into the *S. cerevisiae* haploid strain MT700/9d (both [*PSI*$^+$] and [*psi*$^-$] derivatives: Table 1; Parham *et al.*, 2001), which carried both the *sup35*::*kanMX* allele and the viability-maintaining, single-copy plasmid pYK810 (Kikuchi *et al.*, 1988) bearing the *ScSUP35* and *URA3* genes, or into the [*psi*$^-$] strains MT26.4/3c or /5c carrying the plasmid pDR4 (Table 3). These strains were transformed with the various plasmid constructs carrying the *HIS3* marker and one or other of the different *SUP35* genes. His$^+$ transformants were selected in SD − His medium, and single transformants were then spread onto both solid SD − His medium and YPD medium containing 5-fluoroorotic acid (1 g l^{-1}), selecting for strains that had lost the *URA3*-containing plasmid (Kaiser *et al.*, 1994).

Western blot analysis. Yeast protein extracts were prepared and fractionated into soluble and pelletable (i.e. insoluble) fractions by centrifugation essentially as described by Parham *et al.* (2001). Protein samples were analysed using 10% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose (Sartorius) for Western blot analysis, employing polyclonal antiserum raised against ScSup35p expressed in *E. coli*, essentially as described by Stansfield *et al.* (1995a, b). Anti-rabbit secondary antibody was purchased from Amersham. The ECL reagent (Amersham) was used for detection of bound antibody according to the manufacturer's protocols. Molecular masses of proteins were estimated using pre-stained molecular mass markers (Sigma).

Preparation of yeast ribosomes and post-ribosomal supernatants. Both *S. cerevisiae* and *C. albicans* strains were grown at 30 °C in YPD to a density of 1–2 × 10^7 cells ml^{-1} and harvested by centrifugation. Total cell lysates were then prepared in A buffer (25 mM Tris pH 7·2, 50 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol, 10 mM PMSF) essentially as described by Stansfield *et al.* (1992) and loaded onto a sucrose cushion (15%, w/v, sucrose; 25 mM Tris pH 7·2, 150 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol). After high-speed centrifugation (100000 *g*, 5 h at 4 °C in a fixed-angle rotor), the resulting post-ribosomal supernatant was divided into aliquots and the ribosomal pellet resuspended in A buffer.

Quantification of nonsense suppression levels *in vivo*. Yeast strains were transformed with one or other of the two 'read-through assay' vectors, pUKC815-L or pUKC817-L (Stansfield *et al.*, 1995a). The single-copy vector pUKC815-L carries the *lacZ* gene under the control of the constitutive *PGK* promoter. The read-through vector pUKC817-L has an in-frame premature ochre (TAA) termination codon at the *PGK–lacZ* junction. The degree of nonsense codon suppression was determined essentially as described by Stansfield *et al.* (1995a). Units of β-galactosidase activity were calculated according to Miller (1972). β-Galactosidase activity was expressed as a percentage of the control activity (i.e. that of strains bearing the control vector pUKC815-L).

RESULTS

Isolation and analysis of the *CaSUP35* gene

The *C. albicans SUP35* gene homologue (*CaSUP35*) was identified in a genomic library by its ability to functionally complement the termination phenotype of the *S. cerevisiae sal3-4* allosuppressor allele of the *SUP35* gene (Crouzet *et al.*, 1987). A 2·6 kb *C. albicans* genomic DNA fragment from the single clone identified (plasmid pDR1) was completely sequenced on both strands and the resulting DNA sequence (GenBank accession no. AF020554) identified an open reading frame of 729 residues (Fig. 1a), with a predicted molecular mass of 80·6 kDa. The C-terminal 430 aa sequence (amino acids 300–729) was highly homologous to the corresponding region of *S. cerevisiae* Sup35p (ScSup35p) (79% amino acid identity) as well as to the translation elongation factor eEF1A of *C. albicans* (35% amino acid identity).

Alignment between the ScSup35p and CaSup35p sequences identified the region between amino acids 1 and 143 of the CaSup35p as corresponding to the ScSup35p PrD (Fig. 1a). Although the putative CaSup35p PrD shows little primary amino acid sequence identity to the

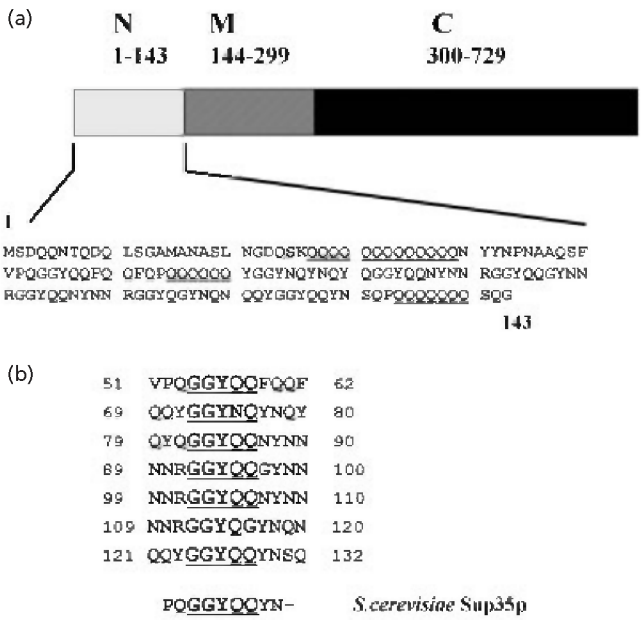

Fig. 1. The *C. albicans* Sup35p protein. (a) Amino acid sequence of the N domain (amino acids 1–143) of CaSup35p deduced from the *CaSUP35* gene sequence (GenBank accession no. AF020554). The three regions identified are equivalent to the N, M and C domains defined in the ScSup35p sequence (Ter-Avanesyan *et al.*, 1993) based on amino acid identity and the position of the indicated Met residues at residues 1 and 144. The three poly(Gln) repeats are underlined. (b) The seven copies of the GGYQQ repeat in the putative N domain of CaSup35p.

Fig. 2. Expression of CaSup35p in *S. cerevisiae* and *C. albicans*. Western blot analysis of clarified cell-free lysates was carried out as described in Methods. Ten micrograms of protein was loaded into each lane and equal loadings were confirmed by Coomassie blue staining. Lanes: 1, *S. cerevisiae* strain BSC783/4a; 2, *S. cerevisiae* strain BSC783/4a transformed with the single-copy Ca*SUP35*-containing plasmid pDR4; 3, an *S. cerevisiae sup35*::*kanMX* disruptant strain (MT26.4/3c) carrying the plasmid pDR4; 4, *C. albicans* strain 2005E grown in yeast (Y) form; 5, *C. albicans* strain 2005E grown in hyphal (H) form. The antibody used to probe the blot was a polyclonal antiserum raised against ScSup35p. Antibody–antigen complexes were detected by chemiluminescence.

ScSup35p PrD, it is similarly rich in Gln and Asn residues (ScSup35p 53/123, 43%; CaSup35p 74/143, 53%). The core motif of the ScSup35p PrD oligopeptide repeat, GGYQQ, is present as five perfect and two imperfect copies in the CaSup35p N domain (Fig. 1b). An additional striking feature of the CaSup35p putative PrD is the presence of three distinct poly(Gln) tracts, the largest containing a run of 13 Gln residues (amino acids 27–39; Fig. 1b).

Hybridization experiments with digested total genomic DNA from *C. albicans* strain 2005E, probed with a 2 kb *Spe*I–*Kpn*I fragment containing the *CaSUP35* gene, demonstrated that *CaSUP35* was present as a single copy sequence (data not shown). Northern blot analysis confirmed that *CaSUP35* encodes a single 2400 nt mRNA transcript (data not shown).

CaSUP35 can provide the essential eRF3 function in *S. cerevisiae*

The ability of the *CaSUP35* gene to supply the essential Sup35p function to an *S. cerevisiae* strain carrying a *sup35*::*kanMX* disruption was tested. The *CaSUP35* gene should be efficiently and authentically translated in *S. cerevisiae* since there are no CUG codons in the coding sequence; the CUG codon is exclusively decoded as Ser rather than Leu in *C. albicans* (Santos & Tuite, 1995). A diploid strain MT26.4 (Table 1), heterozygous for a *sup35*::*kanMX* deletion, was constructed and transformed with pDR4, a *URA3*-based CEN-plasmid carrying the *CaSUP35* gene (Table 3). Viable haploid progeny carrying the *sup35*::*kanMX* disruption were obtained and found to be incapable of growing in the presence of 5-fluoroorotic acid, confirming that their growth depended on retention of the plasmid-borne *CaSUP35* gene. The *sup35*::*kanMX* pDR4-containing haploid progeny showed a weak allosuppressor phenotype (i.e. pink colonies, weak growth in the absence of adenine), indicating that CaSup35p is unable to fully restore efficient translation termination in the absence of ScSup35p. This did not simply reflect a lower steady-state level of the heterologous Sup35p since Western blot analysis of a *sup35*::*kanMX*, pDR4 strain, using an anti-ScSup35p, confirmed the expression of CaSup35p in *S. cerevisiae* (Fig. 2) to a level approximately equivalent to the endogenous ScSup35p. Neither was the allosuppressor phenotype due to the formation of [*PSI*$^+$] since growth of this transformant in 3 mM GuHCl, a treatment known to cure yeast cells of the [*PSI*$^+$] determinant (Tuite *et al.*, 1981), had no effect on the allosuppression phenotype.

To quantify the extent of the termination defect observed in the various strains expressing CaSup35p, the levels of *SUQ5*-mediated ochre suppression were quantified using a *PGK–lacZ* fusion assay (Firoozan *et al.*, 1991; Stansfield *et al.*, 1995a). The results (Table 4) confirm that the single-copy *CaSUP35* gene only partially complemented the termination defect associated

Table 4. Efficiency of nonsense suppression in strains carrying different combinations of *S. cerevisiae* and *C. albicans SUP35* gene alleles

Strain	*SUP35* gene*	Readthrough (%)†
BSC783/4a	*SUP35*	0·82 ± 0·28
BSC783/4a *sal3-4*	*sal3-4*	23·92 ± 8·15
BSC783/4a *sal3-4*	*sal3-4* [*ScSUP35*]	4·19 ± 0·70
BSC783/4a	*SUP35* [*CaSUP35*]	9·66 ± 6·80
BSC783/4a *sal3-4*	*sal3-4* [*CaSUP35*]	8·02 ± 2·47
MT26.4/3c	*sup35*::*kanMX* [*CaSUP35*]	12·85 ± 4·11

* Plasmid-borne genes are shown in square brackets. The plasmid carrying the Sc*SUP35* gene was pYK810 and the plasmid carrying the Ca*SUP35* gene was pDR4, both single-copy, CEN-based plasmids.

† β-Galactosidase activity is expressed as a percentage of the control, i.e. the relevant strain bearing the control vector pUKC815-L. The data are the means ± SD of four independent assays using two different transformants.

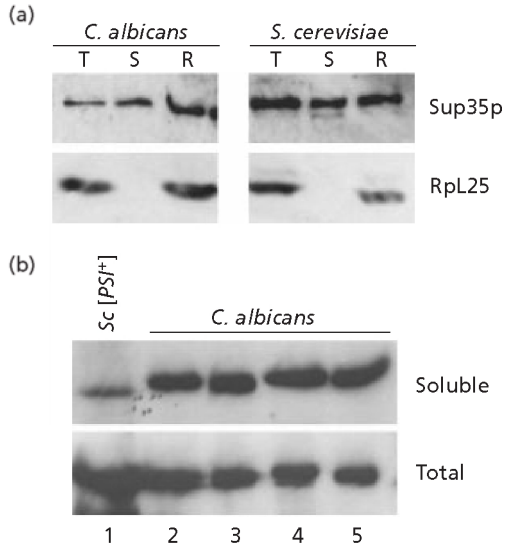

Fig. 3. Subcellular distribution of CaSup35p in *Candida albicans*. (a) Distribution of CaSup35p between the ribosome fraction and the post-ribosomal supernatant fraction. Cell fractions were prepared from cycloheximide-treated *C. albicans* or *S. cerevisiae* and total unfractionated lysates (T), ribosomes (R) and post-ribosomal supernatant (S) samples were generated as described in Methods. Ten micrograms of protein from each sample was subjected to Western blot analysis and probed with either anti-ScSup35p or anti-ribosomal protein L25 antibodies as described in Methods, and antibody–antigen complexes were detected by chemiluminescence. (b) Extent of solubility of CaSup35p in non-cycloheximide-treated strains of *C. albicans*. Samples were prepared essentially as described by Parham *et al.* (2001). Four strains with differing numbers of Gln residues beginning at amino acid 27 are shown: lane 2, strain 2005E (7 Gln); lane 3, W01 (9 Gln); lane 4, strain 81/175 (13 Gln); lane 5, strain J930510 (11 Gln). Lane 1 shows results for *S. cerevisiae* MT700/9d [*PSI*$^+$] for comparison. Total and soluble fractions are shown for each strain.

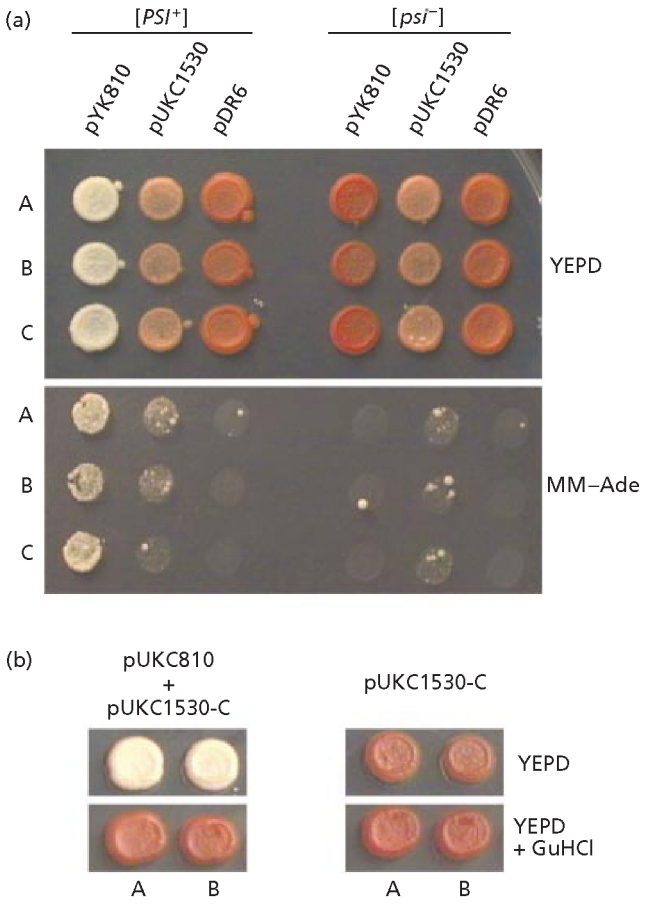

Fig. 4. CaSup35p can functionally replace ScSup35p in *S. cerevisiae*, but cannot be seeded by the endogenous ScSup35p in a [*PSI*$^+$] strain. (a) Either a single-copy plasmid bearing the *ScSUP35* gene (pYK810) or a single-copy (pUKC1530) or multicopy (pDR6) plasmid carrying the *CaSUP35* gene was introduced into a either a [*PSI*$^+$] or a [*psi*$^-$] *sup35*::*kanMX* MT700/9d strain, by plasmid shuffling, as described in Methods. Shown are three independent transformants (A–C) for each, grown on YEPD with a limiting amount of glucose to enhance the colony colour. Note that the colony colour of the pUKC1530 transformants is pink in both strains used whereas the pDR6 transformants are red. (b) Expression of the C domain of CaSup35p in the [*PSI*$^+$] strain MT700/9d either in the presence of the *ScSUP35* gene or after loss of the plasmid pYK810 following plasmid shuffling. The plasmid encoding the CaSup35p C domain is pUKC1530-C.

with both the *sal3-4* allele of *SUP35* (see above) and the *sup35*::*kanMX* disruption. The co-expression of CaSup35p in a strain expressing wild-type ScSup35p also resulted in a detectable decrease in termination efficiency. These data suggest that the CaSup35p interacts less efficiently with components of the endogenous translation termination machinery, most likely eRF1 (Stansfield *et al.*, 1995b).

CaSup35p is ribosome-associated but does not form high-molecular-mass aggregates in *C. albicans*

CaSup35p contains an epitope that is recognized by our anti-ScSup35p polyclonal antibody (Fig. 2; lanes 2–5) and we therefore were able to use this antibody to study the subcellular location of the CaSup35p. Studies with ScSup35p (Didichenko *et al.*, 1991) have shown that when translation elongation is 'frozen' by the addition of cycloheximide, ScSup35p is found associated with the ribosome. We confirmed that this was also the case for CaSup35p in *C. albicans*, with the CaSup35p being clearly present in the ribosomal fraction (Fig. 3a).

Given the known propensity for ScSup35p to form high-molecular-mass aggregates in [*PSI*$^+$] strains of *S. cerevisiae* (Patino *et al.*, 1996; Paushkin *et al.*, 1996) we next determined the state of the CaSup35p in *C. albicans* in non-cycloheximide-treated cells. In this analysis several different naturally occurring clinical strains of *C. albicans* were studied (see below). In each strain, the majority of the detectable CaSup35p was in the soluble fraction, with little if any being detected in the high-molecular-mass fraction (Fig. 3b). This would suggest that these various strains do not contain CaSup35p in a highly aggregated form indicative of the prion state.

Analysis of the CaSup35p levels in both the yeast and hyphal phases of *C. albicans* indicated that this trans-

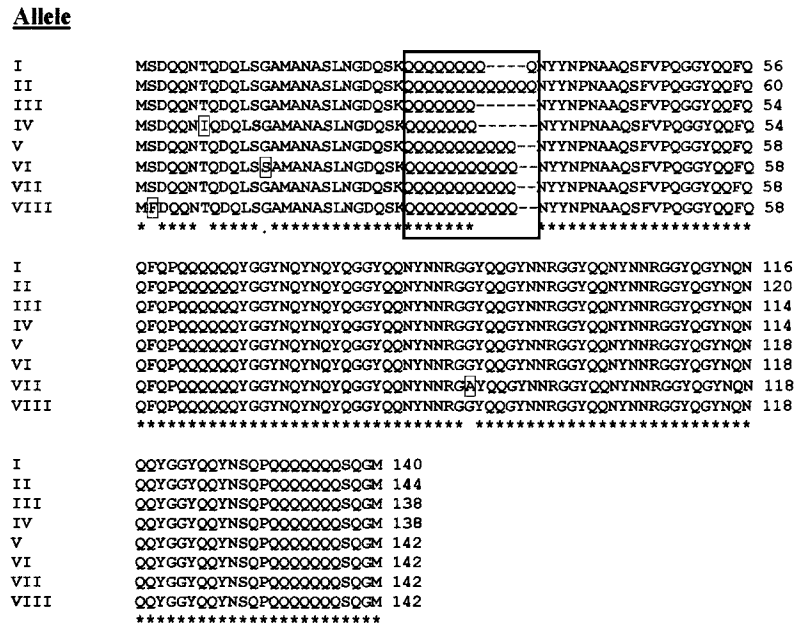

Fig. 5. Sequence polymorphisms within the N domain (amino acids 1–143) of CaSup35p. The polymorphic poly(Gln) repeat is boxed, as are the single amino acid substitutions. Allele numbers correspond to the following strains (see Table 2): I, W01, II, ATCC 10261, 81/175; III, 2005E, WHH1, WHH3, WHH7; IV, WHH4; V, 81/191, 81/192, J930510; VI, 5; VII, 78/011; VIII, IGC 2427.

lation factor is not differentially expressed between the two morphological forms (Fig. 2, lanes 4 and 5). Swoboda *et al.* (1994) reported similar findings for the translation elongation factor eEF-3.

The *CaSUP35* gene does not sustain the [*PSI*$^+$] determinant in *S. cerevisiae*

It has been previously shown by others that overexpression of the CaSUp35p N domain in *S. cerevisiae* does not induce the *de novo* conversion of endogenous ScSup35p to form the [*PSI*] determinant (Santoso *et al.*, 2000; Chien & Weissman, 2001). To determine whether overexpression of the full-length CaSup35p in *S. cerevisiae* might be more effective as a cross-species seeding agent we utilized the strain MT700/9d. This haploid strain carries a *sup35*::*kanMX* disruption, with viability being maintained by the single-copy plasmid pYK810 carrying the wild-type *ScSUP35* gene (see Methods). Starting with either a [*PSI*$^+$] or a [*psi*$^-$] derivative of this strain, in conjunction with a plasmid-shuffling strategy (Parham *et al.*, 2001) we introduced either a single-copy plasmid (pUKC1530) or a multicopy plasmid (pDR6) carrying the full-length *CaSUP35* gene into both strains. Following elimination of the *ScSUP35*-bearing plasmid pYK810, the CaSup35p was unable to induce or sustain the [*PSI*$^+$] phenotype in either strain, even when introduced on a multicopy plasmid (Fig. 4a). In a control experiment, the introduction of the *ScSUP35* gene on plasmid pYK810 maintained [*PSI*$^+$] after plasmid shuffling (Fig. 4a). The weak termination defect (i.e. allosuppression) associated with the pDR4 transformant (see above) was also observed with the pUKC1530 transformant although, as with the pDR4 transformant, this phenotype was not eliminated following growth on 3 mM GuHCl (Tuite *et al.*, 1981) confirming this was not a [*PSI*$^+$]-associated phenotype. In strains carrying the multicopy plasmid pDR6, this allosuppression phenotype was no longer detected. Thus, full-length CaSup35p cannot be seeded by ScSup35p [*PSI*$^+$] aggregates *in vivo* to undergo the necessary conformational change required to sustain Sup35p aggregates (i.e. the [*PSI*] determinant) in *S. cerevisiae*.

We also repeated the experiment using a single-copy plasmid that only expressed the C domain (amino acids 300–729; Fig. 1a) of CaSup35p, i.e. plasmid pUKC1530-C. Post-shuffling, viable Ure$^-$ transformants were obtained, confirming that it is the C domain of CaSup35p that also carries the essential function as for ScSup35p (see above). The resulting transformants clearly had a [*psi*$^-$] phenotype (Fig. 4b; right panel) but no termination defect – as indicated by a weak suppression phenotype – was observed, in contrast to the results with pUKC1530 (Fig. 4a). This would suggest that the M domain of CaSup35p may interfere with the interaction with the endogenous Sup45p (eRF1). When the C domain of CaSup35p was co-expressed with full-length ScSup35p in a [*PSI*$^+$] strain (Fig. 4b; left panel), no antisuppressor phenotype was observed, indicating that the expressed heterologous C domain could not support the [*PSI*$^+$]-related termination defect. Western blot analysis of these strains failed to detect the expressed C domain of CaSup35p, indicating that the epitope recognized by the anti-ScSup35p antibody most likely lies in the N/M domain of CaSup35p.

Natural variation in the N domain of CaSup35p

To determine whether the amino acid sequence of the N domain of CaSup35p was conserved between different strains of this pathogenic fungal species we next PCR-amplified the region of the *CaSUP35* gene encoding amino acids 1–143 from 14 different clinical strains obtained from a variety of sources (Table 2) using a high-fidelity *Taq* polymerase (*Pwo*) with a 3′ to 5′ exo-proofreading activity. Among the 14 strains analysed

eight different *CaSUP35* alleles were observed (Fig. 5). Most significantly, there were four alleles with differing numbers of Gln residues in the largest of the poly(Gln) repeat regions in the N domain (Fig. 1a; amino acids 27–30) with lengths of 7, 9, 11 and 13 residues. The other four variants noted were single amino acid substitutions at positions 2, 6 and 12 in the region N-terminal to the variable-length poly(Gln) repeat region, and at position 93 within one of the copies of the repeated GGYQQ motif.

DISCUSSION

CaSup35p is a functional homologue of ScSup35p

The *C. albicans SUP35* gene encodes a protein showing a significant level of overall amino acid identity to *S. cerevisiae* Sup35p (65 % identity). As would be expected, given this high degree of amino acid sequence conservation, CaSup35p is able to restore viability to an otherwise lethal disruption of the homologous gene in *S. cerevisiae*. The association of CaSup35p with the ribosome confirms the functional equivalence of CaSup35p with ScSup35p. To date, the only known function of Sup35p in yeasts is as a subunit of the translation termination release factor and, as such, it interacts with Sup45p (eRF1) to mediate translation termination at all three stop codons (Stansfield *et al.*, 1995a; Zhouravleva *et al.*, 1995). We have also recently identified a close homologue of Sup45p in *C. albicans* (A. Brandao-Burch & M. F. Tuite, unpublished data). Although mRNA decoding in *C. albicans* is non-standard (Santos & Tuite, 1995), there is no evidence that *C. albicans* decodes any of the three stop codons in any way other than as stop even though such genetic code deviations are relatively common compared to sense codon reassignments.

The ScSup35p molecule appears to have three distinct functional domains, N, M and C. These were originally annotated on the basis of the location of the first three Met residues in the ScSup35p sequence, Met1, Met124 and Met 254 (Kushnirov *et al.*, 1988; Wilson & Culbertson, 1988). Although the equivalent N region of the CaSup35p contains an additional Met residue (Met 15; Fig. 1a), the Met residues at positions 144 and 300 in the CaSup35p sequence correspond to the Met residues used to define the N, M and C regions of ScSup35p. The C domain of ScSup35p (amino acids 254–685) is known to carry the function with regard to translation termination (Ter-Avanesyan *et al.*, 1993) and we show here that the equivalent C domain of CaSup35p also carries the same essential function (Fig. 4b). Both the CaSup35p and the ScSup35p C domains show significant amino acid identity to translation elongation factor eEF1A (Kushnirov *et al.*, 1988; Wilson & Culbertson, 1988; Ter-Avanesyan *et al.*, 1993). While the role of eEF1A is well defined in the translation elongation cycle, the exact role of ScSup35p, and more specifically its essential C domain, in translation termination remains to be established. The presence of GTP-binding domains, which are conserved between eEF1A and Sup35p from both *S. cerevisiae* and *C. albicans*, implies that GTP hydrolysis is required for Sup35p activity although, as with other translation factors with such G domains, this may simply regulate binding of the substrate, which, in this case, would be Sup45p (eRF1) and/or the ribosome.

The failure of the CaSup35p to fully restore efficient translation termination in either an *S. cerevisiae sup35* null mutant or a *sal3-4* mutant, as defined by the weak nonsense suppressor phenotype, is not a consequence of lower cellular levels of the heterologous Sup35p (Fig. 2). Rather, it may simply reflect lower efficiency of binding to Sup45p (eRF1) or another, yet-to-be defined, component of the translation termination machinery. Overexpression of CaSup35p does however restore efficient termination (Fig. 4a), suggesting that a combination of lower efficiency of initial binding between CaSup35p and ScSup45p with the elevated level of CaSup35p in this strain ensures that a level of functional CaSup35p–ScSup45p complex is formed sufficient to allow for efficient termination of translation.

The N domain of CaSup35p

CaSup35p has an N domain of 143 aa which shows many of the sequence features of the well-defined N-terminal prion-forming domain (PrD) of ScSup35p (Tuite, 2000). In particular, there is a high content of Gln and Asn residues (53 %) and a low content of charged residues (5 %). Between the PrD and the essential C domain lies the functionally undefined M domain, whose role in ScSup35p function in termination is dispensable although it remains to be established whether this part of the protein contributes to the prion-like behaviour of ScSup35p. The ScSup35p M domain is 130 aa in length and is highly charged, with Lys and Glu representing 36 % of the residues. The corresponding region in CaSup35p is some 26 residues longer, with 26 % of the residues being Lys and Glu. Alignment between the putative M regions of published fungal Sup35p sequences shows little evidence of evolutionary conservation of primary sequence, although a consistent feature is a high proportion of Lys and Glu residues (data not shown).

Santoso *et al.* (2000) have recently shown that a chimaeric Sup35p containing the NM domain of CaSup35p (amino acids 1–299) and the C domain of ScSup35p could not be incorporated into endogenous ScSup35p prion aggregates in a [*PSI*$^+$] strain. Furthermore, these authors have now shown that this apparent species barrier is probably the consequence of differences in conformation and hence seeding potential (Chien & Weissman, 2001). In our experiments reported here, we demonstrate that full-length CaSup35p is similarly unable to be seeded by the endogenous ScSup35p prion even when overexpressed (Fig. 4a). This would suggest that the lack of the CaSup35p C domain in the recombinant molecules used in the cross-seeding experiments of Santoso *et al.* (2000) did not alter the inter- or intramolecular interactions required for aggregate form-

ation, ruling out the need for an interaction between the CaSup35p N and C domains in prion aggregation. This would in turn rule out a role for the C domain in establishing the 'species barrier' to fungal prion propagation. We have recently shown that replacing the CaSup35p M domain in a hybrid Sup35p molecule with the ScSup35p M domain does not overcome this potential 'species barrier' (C. Resende, S. N. Parham & M. F. Tuite, unpublished data). However, Santoso *et al.* (2000) showed that a CaSup35pNM–GFP fusion protein, when expressed at high levels, was able to form prion-like foci in both [*PSI*$^+$] and [*psi*$^-$] cells with similar kinetics. This finding demonstrates that the CaSup35pNM region can direct self-aggregation *in vivo*, but its polymerization requires no interaction with the endogenous ScSup35p.

Given the similarity in amino acid composition between the PrD of ScSup35p and CaSup35p, and in particular the presence of related repeat sequences (with the 'core' motif of GGYQQ) in both, it is perhaps surprising that the CaSup35p is not seeded by ScSup35p. Santoso *et al.* (2000) demonstrated that replacement of a short region of the CaSup35pN domain with the 19 aa between residues 8 and 26 from the ScSup35pN domain generated a CaSup35pNM–GFP fusion protein whose aggregation could now be seeded by the endogenous ScSup35p. Their data suggest that a region of the N domain of Sup35p N-terminal to, but excluding, the oligopeptide repeats, plays a key role in driving prion conversion of Sup35p. This region shows no amino acid sequence identity between the ScSup35p-PrD and the CaSup35p-PrD, and the corresponding region in the ScSup35p N domain has a significantly higher proportion of Gln and Asn residues than the corresponding CaSup35p region. These differences may be sufficient to inhibit the conformational conversion event even though the heterologous N domain may be able to physically interact via the conserved oligopeptide repeats (see below).

As suggested by the experiments of Chien & Weismann (2001) the failure to efficiently recruit the newly synthesized CaSup35p molecules into the pre-existing ScSup35p aggregates in a [*PSI*$^+$] strain is probably due to the different conformations these two proteins take up in the cell. More specifically, the underlying problem may be the different folding intermediates that predispose the nascent polypeptide chains to conformational conversion *in vivo*. Structure prediction suggests that both the ScSup35p and the CaSup35p N domains are highly flexible and unstructured regions. However, an important requirement for prion establishment and maintenance in *S. cerevisiae* is a dependence upon a certain 'wild-type' level of the molecular chaperone Hsp104 (Chernoff *et al.*, 1995). Thus, in *S. cerevisiae*, the newly synthesized CaSup35p may be unable to access one or more of the components of the protein-folding machinery to which the ScSup35pN domain has been evolutionarily tuned.

One of the most intriguing features of the ScSup35p PrD is the presence of five copies of a 9 aa repeat with a conserved core motif of GGYQQ. This highly repeated core motif is also present in multiple copies in the N domain of the CaSup35p (Fig. 1b). Deletion analysis of the equivalent ScSup35p repeats has led us to propose that these repeats mediate the Sup35p–Sup35p interactions that precede the conformational conversion event that leads in turn to aggregate formation (Parham *et al.*, 2001). Even deletion of one repeat, although not preventing interaction with the wild-type ScSup35p PrD, does prevent interaction with similarly deleted ScSup35p–PrD molecules. That the CaSup35p is unable to interact with the wild-type ScSup35p to form aggregates would suggest that multiple copies of the GGYQQ motif are not sufficient for the important intermolecular interactions required for prion propagation. However, it might be that the conformational conversion of the oligomeric intermediates (Serio *et al.*, 2000) rather than the intermolecular interactions *per se* is the step that is blocked or at least less efficient. The transient intramolecular interactions between the CaSup35p PrD and the molecular chaperone Hsp104 that are essential for [*PSI*$^+$] maintenance in *S. cerevisiae* (Chernoff *et al.*, 1995) may therefore not be optimal for efficient propagation of a CaSup35p-based prion in the absence of the homologous folding factor.

Polyglutamine repeat expansion in the N domain of CaSup35p?

One feature of the CaSup35p that differentiates it from the ScSup35p sequence is the presence of several poly(Gln) repeat regions. Expansion of such poly(Gln) repeats has been identified with a number of inherited human diseases, in particular Huntington's disease, fragile X syndrome and myotonic dystrophy (reviewed by Zoghbi & Orr, 2000), and this is due to the underlying protein having an increased propensity to aggregate in the brain. Our finding that there is a significant level of polymorphism in the length of one (but not all) such tracts in the CaSup35p N domain (Fig. 5) raises the interesting possibility that CaSup35p aggregation may be modulated by a different mechanism to ScSup35p (which has no such poly(Gln) repeat regions in the PrD). It remains to be established whether or not changes in the Gln repeat number do alter the aggregation behaviour of CaSup35p in *C. albicans*. It should be noted that the subcellular fractionation analysis of strains carrying the different *CaSUP35* alleles (Fig. 3) failed to detect any differences in the levels of solubility of the CaSup35p between the strains although the centrifugation steps we employ only pellet material in excess of 190S (F. Ness & M. F. Tuite, unpublished data). We also note that the variable poly(Gln) repeat region lies within the same region as implicated in the species barrier of yeast prion propagation (Santoso *et al.*, 2000).

The mechanism by which poly(Gln) repeats expand is still far from clear although meiotic gene conversion and/or unequal crossing-over between two homologous chromosomes has been widely suggested (Berg *et al.*, 2000; Jakupciak & Wells, 2000). It is important to note

that *C. albicans* is apparently unable to undergo meiosis, lacking many of the genes essential for meiosis in *S. cerevisiae* (Tzung *et al.*, 2001). This would suggest that the mechanism of poly(Gln) repeat expansion in *C. albicans* may occur via a non-meiotic event, either through unequal crossing-over between the two sister chromatids of a chromosome during mitosis or due to replication slippage (reviewed by Moore *et al.*, 1999). None of the strains we studied showed clear evidence of heteroallelism at the *CaSUP35* locus since all DNA sequences of the PCR-generated fragments were homogeneous, although were polymorphisms to exist within the primer binding site(s), this might bias such an analysis in favour of one allele. The significance of this high-frequency variation in the length of the poly(Gln) repeat in CaSup35p may be in the rapid evolution of a directed mechanism of protein aggregation akin to the prion-like behaviour of the *S. cerevisiae* homologue. It now remains to be established whether any of the identified alleles of the *CaSUP35* gene encode a form of CaSup35p that is able to behave as a prion in *C. albicans*.

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