Genetic Study of Interactions Between the Cytoskeletal Assembly Protein Sla1 and Prion-Forming Domain of the Release Factor Sup35 (eRF3) in Saccharomyces cerevisiae

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ABSTRACT

Striking similarities between cytoskeletal assembly and the “nucleated polymerization” model of prion propagation suggest that similar or overlapping sets of proteins may assist in both processes. We show that the C-terminal domain of the yeast cytoskeletal assembly protein Sla1 (Sla1C) specifically interacts with the N-terminal prion-forming domain (Sup35N) of the yeast release factor Sup35 (eRF3) in the two-hybrid system. Sla1C and several other Sup35N-interacting proteins also exhibit two-hybrid interactions with the poly-Gln-expanded N-proximal fragment of human huntingtin, which promotes Huntington disease-associated aggregation. The Sup35N-Sla1C interaction is inhibited by Sup35N alterations that make Sup35 unable to propagate the [PSI+] state and by the absence of the chaperone protein Hsp104, which is essential for [PSI+] propagation. In a Sla1δ background, [PSI+] curing by dimethylsulfoxide or excess Hsp104 is increased, while translational readthrough and de novo [PSI+] formation induced by excess Sup35 or Sup35N are decreased. These data show that, in agreement with the proposed function of Sla1 during cytoskeletal formation, Sla1 assists in [PSI+] formation and propagation, but is not required for these processes. Sla1δ strains are sensitive to some translational inhibitors, and some sup35 mutants, obtained in a Sla1δ background, are sensitive to Sla1, suggesting that the interaction between Sla1 and Sup35 proteins may play a role in the normal function of the translational apparatus. We hypothesize that Sup35N is involved in regulatory interactions with intracellular structural networks, and [PSI+] prion may be formed as a by-product of this process.
reversing stress-induced aggregation damage to cellular proteins (Parsell et al. 1994; Lindquist et al. 1995), plays an important role in [PSI] propagation: Hsp104 overproduction or inactivation cures cells of [PSI] (Chernoff et al. 1995).

It has been suggested that prion propagation is mediated or facilitated by a nucleated polymerization mechanism, which is somewhat analogous to the formation of cytoskeletal filamentous structures (Lansbury and Caughey 1995). Both mammalian (Prusiner et al. 1983) and yeast (King et al. 1997; Taylor et al. 1999) prion proteins or prion-forming domains are able to undergo polymerization in vitro, resulting in the formation of amyloid-like fibers. Cell-free, self-seeded formation of the Sup35 aggregates can be reproduced in several subsequent cycles, thus mimicking in vivo propagation of [PSI] prion (Paushkin et al. 1997; De Pace et al. 1998). An apparent similarity between prion propagation and the assembly of highly ordered structures, such as cytoskeletal fibers, leads to the suggestion that these processes may be related to each other.

Yeastsup35 protein consists of three distinct regions, designated as Sup35N, Sup35M, and Sup35C (Kushnirov et al. 1988, 1990). While the amino acid sequence of Sup35C is conserved from yeast to human, the regions of Sup35N and Sup35M are variable in evolution (Hoshino et al. 1989; Kushnirov et al. 1990; Chernoff et al. 1992b; Jean-Jean et al. 1996). The Sup35C region is required and sufficient for cell viability and translational termination function (Ter-Avanesyan et al. 1993). The biological role of the Sup35N and Sup35M domains remains unknown. Deletion of the SUP35N and SUP35M domains does not have any detectable effect on growth (Ter-Avanesyan et al. 1993). However, the Sup35N domain is responsible for [PSI] propagation (Doel et al. 1994; Ter-Avanesyan et al. 1994), overproduction-induced translational readthrough (Ter-Avanesyan et al. 1993) and [PSI] appearance (Der- katch et al. 1996), and in vitro polymerization (Glover et al. 1997; King et al. 1997). The Sup35N domain contains oligopeptide repeats, rich in Pro, Gly, and Gln residues and similar to those found in the mammalian PrP (Cox 1994; Kushnirov et al. 1995; Tuite and Lindquist 1996), and a Gln-rich stretch similar to the one present in some neural inclusion-associated proteins, such as huntingtin (De Pace et al. 1998). It has been shown that polyGln-expanded fragments of mutant huntingtin substitute for the Sup35N Gln-rich stretch in promoting Sup35 aggregation (De Pace et al. 1998). This suggests that the molecular bases for the polymerization of Sup35[psi]+ and the aggregation of the mutant huntingtin are similar.

Here, we identify several yeast proteins that interact with the Sup35N domain in a two-hybrid assay. These proteins also exhibit interactions with the polyGln-expanded huntingtin, further underlining the similarities between these two systems. One of the Sup35N-interacting proteins, Sla1, has been investigated in more detail. This protein has previously been suggested to assist in nucleation of the cortical actin microfilaments (Holzman et al. 1993). Our data confirm genetic interactions between Sla1 and Sup35 and show that the formation and stability of [PSI] prion are affected in yeast strains lacking Sla1. This provides the first experimental evidence demonstrating that in vivo molecular processes leading to [PSI] prion formation and assembly of the actin cytoskeleton involve overlapping sets of protein “helpers.”

### Materials and Methods

**Yeast strains:** Strains used in this study are listed in Table 1. OT56 (also called [PSI]+7-74-D694) and OT60 (also called [psi-] 7-4-D694) are isogenic [PSI+] and [psi-] strains, respectively, which have previously been described (Derkatch et al. 1996, 1997). GT17 is a [psi- pin] derivative of OT56, obtained by guanidine hydrochloride (GuHCl) treatment as described previously (Derkatch et al. 1997). GT88 and GT89 are OT60 and OT56 derivatives, respectively, which bear sla1Δ:HIS3 disruptions, constructed by PCR-mediated gene replacement (Baudin et al. 1993) as described below. GT83 is a [psi- pin] derivative of GT89, obtained by GuHCl treatment. The strain PA-P3532 (also called OT61) and its derivatives 66-8A-P3532 (OT64) and 68-8A-P3532 (OT65), containing sup35 and sup45 mutations, respectively, were provided by S. G. Inge-Vechtomov (Inge-Vechtomov et al. 1988a).

The strain P69-4A (James et al. 1996) used as a recipient in the two-hybrid assays was kindly provided by P. James. We have identified P69-4A as [psi-] in a genetic cross to the tester strain bearing the dominant tRNA suppressor SUP16 and UAA allele ade2-1, which is suppressed by SUP16 only in the presence of [PSI]. The MATα derivative of P69-4A was generated by inducing the mating type switch in the presence of the plasmid YrpHO. The hsp104::URA3 derivative of the strain P69-4A was generated by disrupting the HSP104 gene with the URA3 gene as described below. The spontaneous Ura− derivative of this strain, apparently originated from conversion of the wild-type URA3 allele within HSP104 locus into the endogenous mutant ura3 allele, was selected on the medium containing 5-fluoroorotic acid (5-FOA), which prevents growth of Ura− cells (Kaiser et al. 1994).

**Plasmids:** The plasmids pEMBL-SUP35 and pEMBL-SUP35-ΔBal, described previously as pEMBL-SUP2 and pEMBL-SU2-ΔBal, respectively (Ter-Avanesyan et al. 1993), are pEMBL vectors (Cesareni and Murray 1987) derivatives bearing the complete SUP35 gene and the N-terminal 154 codons of the SUP35 gene, respectively, under the normal SUP35 promoter. These plasmids also contain the 2μm DNA replicator and yeast-selectable markers URA3 and LEU2-Δ. The centromeric URA3 plasmid CEN-GAL-SUP35, described previously (Derkatch et al. 1996), contains SUP35 under the galactose-inducible (GAL) promoter. The centromeric URA3 plasmid pYCH-U2, which is a pFL38 (Bonneaud et al. 1991) derivative bearing the complete SUP35 gene under its normal promoter, has been described earlier (Derkatch et al. 1997). Plasmid pFL38-SUP35-PNM2, which differs from pYCH-U2 in that it contains a PNM 2 mutation (Gly58Asp), has been constructed by inserting the 3.4-kb XbaI fragment of pSM128 (Doel et al. 1994) into pFL38. The plasmid pRS316GAL-SUP35N was constructed by inserting the 0.35-kb Smal-EcoRV fragment of CEN-GAL-SUP35, which contains the N-terminal 113 codons of the
**TABLE 1**

**Saccharomyces cerevisiae** strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype/ description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT56 ([PSI-]) 7-74-D694</td>
<td>MATa adel-14$<em>{uga}$ his3 leu2 trp1-289$</em>{ura}$ ura3 [PSI+]</td>
<td>Derkatch et al. (1996)</td>
</tr>
<tr>
<td>OT60 ([psi-])-74-D694</td>
<td>MATa adel-14$<em>{uga}$ his3 leu2 trp1-289$</em>{ura}$ ura3 [psi-]</td>
<td>Chernoff et al. (1995)</td>
</tr>
<tr>
<td>GT17</td>
<td>MATa adel-14$<em>{uga}$ his3 leu2 trp1-289$</em>{ura}$ ura3 [psi-]</td>
<td>Derkatch et al. (1997)</td>
</tr>
<tr>
<td>GT88</td>
<td>slal$_{Δ}$::HIS3 derivative of OT60</td>
<td>This study</td>
</tr>
<tr>
<td>GT89</td>
<td>slal$_{Δ}$::HIS3 derivative of OT56</td>
<td>This study</td>
</tr>
<tr>
<td>GT83</td>
<td>GuHCl-cured [psi-] derivative of GT89</td>
<td>This study</td>
</tr>
<tr>
<td>OT61 (8A-P3532)</td>
<td>MATa adel-14$_{uga}$ his3 mat13-A1 [psi-]</td>
<td>Inge-Vechtomov et al. (1988a)</td>
</tr>
<tr>
<td>OT64 (66-BA-P3532)</td>
<td>MATa adel-14$<em>{uga}$ his3 mat13-A1 sup$</em>{45}$</td>
<td>Inge-Vechtomov et al. (1988a)</td>
</tr>
<tr>
<td>OT65 (68-BA-P3532)</td>
<td>MATa adel-14$<em>{uga}$ his3 mat13-A1 sup$</em>{35}$</td>
<td>Inge-Vechtomov et al. (1988a)</td>
</tr>
<tr>
<td>PJ69-4A</td>
<td>MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4$<em>{Δ}$ gal180$</em>{Δ}$GAL2-ADE2 LYS2::GAL1-HIS3 mat2::GAL7-laet2 [psi-]</td>
<td>James et al. (1996)</td>
</tr>
<tr>
<td>GT50</td>
<td>MAT derivative of PJ69-4A</td>
<td>This study</td>
</tr>
<tr>
<td>GT74</td>
<td>hsp104::URA3 disruption derivative of PJ69-4A</td>
<td>This study</td>
</tr>
<tr>
<td>GT75</td>
<td>Spontaneous Ura+ derivative of GT74</td>
<td>This study</td>
</tr>
</tbody>
</table>

SUP35 gene, into pRS316GAL (Liu et al. 1992) cut with Smal. The resulting vector contains SUP35N under the control of the GAL promoter.

Plasmid pYS104 (Sanchez et al. 1992; Chernoff et al. 1995) is a derivative of the centromeric vector pRS316 (Sikor ski and Hiet er 1989), which bears the HSP104 gene under its normal promoter. Centromeric plasmid pGAL104-URA3 (Sanchez et al. 1993), kindly provided by S. Lindquist, contains the HSP104 gene under the galactose-inducible GAL110 promoter. The plasmid p85-HIS3, which contains the Saccharomyces cerevisiae H3 gene inserted into the pBluescript KS I (+) polylinker (Stragatenge, Lajolla, CA), was constructed by J. Kumar. The centromeric URA3 plasmid YCP-SLA1, which is pRS416 (Sikor ski and Hiet er 1989) bearing the SLA1 gene under its endogenous promoter, was kindly provided by R. Reid. Plasmid pFL44-SLA1 was constructed by inserting the XhoI-HindIII 4.4-kb fragment of YCP-SLA1, which includes the complete SLA1 gene under its endogenous promoter, into the 2µm-based plasmid pFL44 (Bonneau et al. 1991) cut with Sall and HindIII.

**Two-hybrid constructs:** Plasmids pAS1, which bears the GAL 4 DNA-binding domain (GAL4$_{DNA}$) under the ADH promoter and the TRP1 marker, and pACT2, which bears the GAL 4 activator domain (GAL4$_{ACT}$) under the ADH promoter and the LEU2 marker (Durf ee et al. 1993), were kindly provided by S. Eld elde. These plasmids allow for the fusion of the gene fragments of interest to the C termini of the corresponding GAL 4 domains. The two-hybrid library, kindly provided by S. Eld elde, was based on the genetically engineered lambda phage, which spontaneously produces pACT-based plasmids (LEU2 ADH::GAL4$_{ACT}$) by intramolecular recombination, once propagated in the BNN122 strain of Escherichia coli. These plasmids contain inserts fused to the GAL4$_{ACT}$ domain at the XhoI site. The library clone bearing the SLA1C fragment was designated pACT-SLA1C. The plasmid pACT was reconstituted from one of the library clones by cutting the XhoI insert off and religating the vector. It differs from pACT2 in the sequence of the polylinker region following GAL4$_{DNA}$... Plasmids pSE1111 and pSE1112, which bear GAL4$_{ACT}$, SNF1 and GAL4$_{DNA}$-SNF4 chimeric constructs, respectively (Durf ee et al. 1993), and were used as a positive control in two-hybrid experiments, were kindly provided by S. Eld elde. Plasmids pG4BD-0, pG4BD-1, and pG4BD-2, bearing the TRP1 marker and the GAL4$_{DNA}$ under the ADH promoter, were kindly provided by R. Brazas. These plasmids allow the fusion of the gene fragment of interest to the N terminus of the GAL4$_{DNA}$ domain.

Two-hybrid plasmids bearing the S. cerevisiae SUP35 fragments, which have been constructed for this study, are shown on Figure 1A. Plasmids pAS1-SUP35N and pACT2-SUP35N were constructed by inserting the 0.35-kb Smal-EcoRI fragment of CEN-GAL-SUP35, which contains the first 113 codons of the SUP35 gene, into pAS1 or pACT2, respectively, cut with BamHI and blunt-ended with the large (Klenow) fragment of DNA polymerase I. The resulting constructs contain the SUP35N region fused in frame to the C terminus of the GAL4$_{DNA}$ (pAS1-SUP35N) or GAL4$_{ACT}$ (pACT2-SUP35N). The plasmid pG4BD-SUP35N, which contains the SUP35N region fused in frame to the N terminus of GAL4$_{DNA}$, was constructed by inserting the 0.4-kb BamHI-EcoRI fragment of pACT-SUP35 into pG4BD-0 cut with BamHI and Smal. (Plasmid pACT-SUP35 contains the 1.5-kb EcoRI-Sall fragment of CEN-GAL-SUP35, inserted into pACT.)

The plasmid pAS1-SUP35N-Δ22/69 was constructed by digesting pAS1-SUP35N with BstEII and self-ligating, resulting in a 144-bp in-frame deletion, corresponding to the region between amino acid (aa) positions 22 and 69. The plasmid pAS1-SUP35N-PNM2 was constructed by inserting the 144-bp BstEII fragment of pSM128 (Dovel et al. 1994) bearing the PNM2 (Gly58Asp) substitution into BstEII-cut pAS1-SUP35N-Δ22/69.

The plasmid p56G (Hoshino et al. 1989), provided by S. Hoshino and Y. Kikuchi and containing the entire human SUP35Hs (GST1-Hs) clone, served as a donor of the human SUP35 gene fragment. The 0.4-kb BamHI-PvuII fragment of p56G bearing the N-proximal region of the human SUP35Hs from aa position 2 to 135 was obtained by complete digestion with BamHI and incomplete digestion with PvuII and inserted in frame into pACT, which was cut with XhoI, blunted using T4 DNA polymerase, and subsequently cut with BamHI. The resulting plasmid was called pACT-SUP35NHs. The pRS316GAL-SUP35Pm plasmid (Y. Chernoff and G. Newman, unpublished results) contains the entire coding sequence of the Pichia manihotica SUP35 gene (Kushnirov et al. 1990) obtained by PCR from pTR30 (Kushnirov et al. 1990).
Figure 1.—Two-hybrid interactions in the strain PJ69-4A. (A) Two-hybrid plasmids used in this study. The plasmid pAS1-SUP35N contains the SUP35N fragment, which includes the first 113 codons of SUP35 fused to the C terminus of the GAL4 DNA-binding domain (GAL4_{DNA}); the plasmid pG4BD-SUP35N contains SUP35 fused to the N terminus of GAL4_{DNA}. The plasmid pACT-SLA1C, which has been recovered from the library screen (see text), contains the C-terminal 411 codons of SLA1 fused to the C terminus of GAL4_{ACT}. The plasmids pAS1-HUNQ20 and pAS1-HUNQ53 contain the wild-type huntingtin fragment bearing a stretch of 20 CAG (Gln) codons, or the mutant huntingtin fragment bearing an expanded stretch of 53 CAG codons, respectively, fused to the C terminus of GAL4_{DNA}. All plasmids contain the 2 μm DNA origin of replication (2 μm ori), the alcohol dehydrogenase promoter (P_{ADH}), and the HA protein tag. Restriction sites are designated as follows: B, BamHI; EV, EcoRV; S, Sall; Sm, SmaI; X, XhoI. Only sites used in the construction procedures are shown. (B and C) Two-hybrid interactions that involve SUP35N or huntingtin fragments. Sup35N, HunQ20, and HunQ53 were fused to the C terminus of Gal4_{DNA}; HunQ20 + Sla1C, Reg1, Eno2, and Eft2 were fused to the C terminus of Gal4_{ACT}. The plasmid containing GAL4_{ACT} alone is shown as a control. Two-hybrid interaction was observed as GAL2-ADE2 activation detected by growth on 2Trp-Leu-Ade media. (D) Two-hybrid interactions between Sup35N and Sla1C do not depend on the position of SUP35N in the chimeric construct. Growth was tested on 2Trp-Leu media; two-hybrid interaction was observed as GAL2-ADE2 and GAL1-HIS3 activation detected as growth on 2Trp-Leu-His and 5 μm AT media, respectively. Similar results were obtained when Reg1, Eno2, and Eft2 constructs were used instead of Sla1C (not shown).

Both plasmids pBTM116 CAG20 and pBTM116 CAG53A, kindly provided by Dr. E. Wanker (Scherzinger et al. 1997), contain exon 1 of the Huntingtin gene, corresponding to aa positions 1–90 of the published Huntingtin open reading frame (ORF) (GenBank accession no. L27350; Scherzinger et al. 1997), with 20 and 53 CAG (Gln) repeats, respectively. The 0.27-kb Sall-BamHI fragment of pBTM116 CAG20 or the 0.37-kb Sall-BamHI fragment of pBTM116 CAG53 were in-
sented into pAS1 cut with Sall and BamH1 to construct pAS1-HUN20 or pAS1-HUNQ53, respectively (see Figure 1A).

The plasmid WMpVZ1-PrP, kindly provided by Dr. R. Petersen and containing the entire human Prp-p gene (129Met allele) ORF, served as a donor of the Prp-p sequence. The 0.4-kb EcoRI-Pst fragment of WMpVZ1-PrP, coding for the N-terminal 116 aa of PrP, was first inserted into pBluescript KS II (++) to construct pBS-PrP. Then, the 0.4-kb HindIII-BamH1 of pBS-PrP was inserted in frame into pG4BD-0 to construct pG4BD-0-PrP.

Genetic and microbiological techniques: Standard yeast media and genetic techniques were used (Kaiser et al. 1994). Yeast cultures were incubated at 30°C unless specified otherwise.

Quantitative assays for [PSI+] curing by GuHCl or dimethylsulfoxide (DMSO) were performed as follows. Yeast cultures were grown in YPD medium at 25°C with shaking up to approximately 10^8 cells/ml and inoculated at 10^6 cells/ml into fresh YPD medium containing the curing reagent (5 mm GuHCl or 10% DMSO). Cultures were incubated at 25°C with shaking. Alliquests were taken after specific periods of time and plated onto YPD plates. [PSI+] (white) and [psi-] (red) colonies were counted after 3 days of incubation at 30°C followed by 2 days of incubation at 4°C to improve color diagnostics for [PSI].

Quantitative assays for [PSI+] curing by overproduced Hsp104 were performed as follows. Yeast strains were transformed with the plasmid pGAL104-Ura3. Individual transformants were grown at 25°C up to 10^6 cells/ml in the standard −Ura glucose-based synthetic medium, which is selective for plasmid-containing cells, and inoculated at 25°C × 10^5 cells/ml into −Ura medium in which 2% galactose and 2% raffinose were substituted for glucose. The GAL::HSP104 construct is induced in the galactose/raffinose medium as verified by Western blots (not shown). Cultures were incubated at 25°C with shaking. Alliquests were taken after specific periods of time and plated onto −Ura/glucose plates. Plates were incubated for 3 days at 25°C and velveteen replica plated onto YPD plates. [PSI+] and [psi-] colonies were scored by color after 1 day of incubation at 30°C followed by 2 days of incubation at 4°C, as described above.

Omnipotent suppressor mutants were selected as simultaneous revertants of the mutations ade1-14 (UGA) and trpl-289 (UAG) growing on the medium lacking both adenine and tryptophan. As described previously (Inge-Vechtomov et al. 1988a; Cheroff et al. 1996), this procedure yields almost exclusively mutations in the Sup35 and Sup45 genes. Suppressor mutants were selected at 25°C and then checked for their ability to grow at 30°C and 37°C. Allelism of the recessive sup35 and sup45 mutants was determined by the ability of diploids obtained in genetic crosses to the tester strains OT64 (sup35) and OT65 (sup45) to suppress a homozigous ade1-14 mutation, as described (Inge-Vechtomov et al. 1988a).

Gene transplacements: The slaΔ::HIS3 disruptions were constructed by direct PCR-mediated transplacement as described (Baudin et al. 1993). Plasmid pBS-HIS1 was used as a template. PCR primers SLAG1-HIS3-PRO (5'—GACGAGAATGTGTATATACAAAAGA GCTAGAGTATGACCTCTTGGCCT from the Sup35C domain (UGA) and SLAG1-HIS3-TERM (5’ACAAGCTTCATTAATCTAGAATCCAAACGG), which are complementary to the 5' and 3' flanking regions of the SLA1 gene, respectively, were used for PCR analysis. The hsp104 disruption was constructed by substituting the 1.2-kb Apal-BglII piece of the Hsp104 gene by the URA3 gene in the opposite orientation, as described previously (Cheroff et al. 1995). The plasmid-borne hsp104::URA3 allele was substituted for the wild-type HSP104 allele by using the standard one-step gene transplacement procedure (Kaiser et al. 1994). The resulting disruptants were verified by both Southern and Western analyses (not shown).

Molecular biology techniques and materials: Standard protocols were used for DNA isolation, electrophoresis, fragment purification, restriction digestion, and PCR (Sambrook et al. 1989). The thermal cycler was purchased from ERICOMP (San Diego). Restriction enzymes were purchased from New England Biolabs (Beverly, MA) and GIBCO BRL. Taq polymerase was purchased from Promega (Madison, WI) and GIBCO BRL. The DNA probe for Southern hybridization was labeled by Gene Images random prime labeling module (Amersham, Arlington Heights, IL) according to the manufacturer’s procedure. Southern hybridization was performed by using the chemiluminescent probe according to the Gene Images CDP-Star detection protocol from Amersham. DNA sequencing was performed at the Molecular Genetics Instrumentation Facility, University of Georgia (Athens, GA). Hsp104-specific and Sup35-specific antibodies were kindly provided by S. Lindquist. Slal-specific antibodies were kindly provided by D. Drubin. Protein extracts were prepared from yeast cells that had been lysed by vortexing with glass beads in 25 mm Tris-HCl, pH 7.5 + 0.2 mNaCl + 1 mm EDTA + 0.5 mm dithiothreitol + 2 mm phenylmethylsulfonyl fluoride + 5% glycerol. Aggregated (insoluble) Sup35 protein was identified by centrifugation as described (Patino et al. 1996) with slight modifications (Newnam et al. 1999). Western blotting, reaction to primary and secondary antibodies, and detection were performed according to the chemiluminescent procedure as described in the Amersham protocols.

RESULTS

Two-hybrid search for the Sup35N-interacting proteins: Plasmid pAS1-SUP35N (Figure 1A) contains the N-terminal 113 codons of the Sup35N gene fused in frame to the C terminus of the GAL4 DNA domain. This piece of the Sup35N has been previously shown to be able to maintain the [PSI+] state when expressed separately from the Sup35C domain (Terasvany et al. 1994). The pAS1-SUP35N was used as a “bait” to search for DNA clones, products of which interact with the Sup35N domain (see Figure 1B). The strain PJ69-4A, bearing pAS1-SUP35N, was transformed with the pACT-based library, which contains yeast DNA clones fused to the GAL4 DNA domain. Transformants were selected on −Trp-Leu medium and replica plated onto −Trp-Leu-Ade medium. Ade+ transformants, in which GAL2-ADE2 construct is activated, were chosen for further analysis. To eliminate mutations that activate transcription of the

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ADE2 gene in a Gal4-independent fashion, these transformants were screened for activation of the GAL1-HIS3 construct, resulting in growth on –Trp-Leu-His medium in the presence of 5 mm aminotriazole (AT). Positive clones were cured of the pAS1-SUP35N plasmid and crossed to the MATα derivative of P69-4A, bearing the plasmid pSE1112, which contains the GAL4DNA-SNF4 fusion (Durfee et al. 1993). Those activating the GAL2- ADE2 construct in combination with pSE1112 were regarded as false positives and removed from further analysis.

The remaining DNA clones were isolated from yeast transformants, purified through E. coli, and retransformed individually into P69-4A derivatives bearing either pAS1-SUP35N plasmid or pG4BD-SUP35N plasmid. The plasmid pG4BD-SUP35N (Figure 1A) contains the same region of the SUP35 gene as pAS1-SUP35N, but it is attached in frame to the N terminus of the GAL4DNA domain. We observed that the location of the Sup35N domain within the chimeric construct could affect the efficiency of the two-hybrid interaction. For example, efficient intermolecular two-hybrid interactions between Sup35N domains were detected reproducibly only when at least one of these domains was located at the N terminus of the chimeric protein (P. Bailleul and Y. Chernoff, unpublished data). For further analysis, we chose DNA clones that exhibit interactions with both SUP35N constructs (the C-terminal, pAS1-SUP35N, and the N-terminal, pG4BD-SUP35N). These clones were partially sequenced, and the corresponding genes were identified according to the BLAST search in the Yeast Genome Database. Among a total of 15,000 library clones analyzed, 4 nonoverlapping DNA clones were identified whose products specifically interacted with both SUP35N-containing constructs (see Table 2). These include the following: translation elongation factor EF-2; two proteins involved in control of glucose metabolism, Reg1 and Eno2; and the C-terminal portion (411 aa residues) of the cytoskeletal assembly protein Sla1. The SLA1C clone was studied in more detail.

**Specificity of the Sup35N-Sla1C interaction:** The Sla1C domain is highly hydrophobic, possesses a high percentage of Pro and Gly residues, and contains oligopeptide repeats (Holzman et al. 1993), similar to those found in Sup35N and PrP (Cox 1994; Kushnirov et al. 1995). One could suggest that the Sup35N-Sla1C two-hybrid interaction is a result of nonspecific hydrophobic association of protein regions of similar amino acid composition. To check this, we analyzed Sla1C interactions with the following proteins or protein domains: (1) Sup35 homologue from the distant yeast species, P. methanolica; (2) Sup35N-corresponding region from Homo sapiens; (3) the N-terminal half of the human PrP protein. All these proteins have a similar amino acid composition to the S. cerevisiae Sup35N, and both Pichia Sup35 and human PrP contain oligopeptide repeats. However, the Sup35N sequences of both Pichia and human exhibit very little homology with the Sup35N of S. cerevisiae (Hoshino et al. 1989; Kushnirov et al. 1990; Chernoff et al. 1992b; Jean-Jean et al. 1996), and no unambiguous homology between the Sup35N and PrP was detected. The protein fragments, listed above, were fused to the Gal4DNA (see materials and methods) and screened against pACT-SLA1C. No interaction between Sla1C and any of these proteins was detected (not shown), confirming that Sla1C is specifically interacting with S. cerevisiae Sup35N.

We also checked two-hybrid interactions between the Sla1C and the Gln-rich N-terminal fragment of human huntingtin, a protein associated with Huntington disease. The wild-type huntingtin fragment containing a stretch of 20 CAG (Gln) codons did not interact with Sla1C. However, the fragment of mutant (Huntington disease-associated) huntingtin that contains a poly-CAG stretch expanded to 53 codons did exhibit two-hybrid interaction with Sla1C, as well as with other Sup35N-interacting proteins (Figure 1C). This result suggests that poly-Gln-expanded huntingtin possesses protein-interacting patterns that are similar to those of the Sup35 prion-forming domain. This is in agreement with previous observations demonstrating the ability of the poly-Gln-expanded huntingtin fragments to promote Sup35 aggregation in fusion constructs (De Pace et al. 1998).

**[PSI] no more** mutations affect Sup35N-Sla1C interaction: Next we asked if the Sup35N mutations that affect the ability of the Sup35N domain to undergo intermolecular interactions involved in [PSI] propagation would affect the interaction between Sup35N and

| TABLE 2 |
|-----------------|--------|--------|-----------------|-----------------|
| YDR38SW        | EFT2   | 1.4    | 1               | Translation elongation factor 2 | Perentesis et al. (1992) |
| YHR174W        | ENO2   | 1.2    | 3               | Enolase            | Cohen et al. (1986)    |
| YDR02BC        | REG1   | 1.4    | 1               | Positive regulator in glucose metabolism | Naik et al. (1997) |
| YBL007C        | SLA1   | 1.3    | 2               | Cytoskeletal assembly protein (C-terminal domain) | Holtzman et al. (1993) |
| **Gene product description** | **Reference** |
| Translation elongation factor 2 | Perentesis et al. (1992) |
| Enolase | Cohen et al. (1986) |
| Positive regulator in glucose metabolism | Naik et al. (1997) |
| Cytoskeletal assembly protein (C-terminal domain) | Holtzman et al. (1993) |
Sla1C. Two “[PSI] no more” mutations were tested: sup35Δ22/69, a deletion of the region between amino acid positions 22 and 69 (Ter-Avanesyan et al. 1993), and SUP35-PNM2, a Gly58Asp substitution (Doel et al. 1994). The Δ22/69 deletion makes Sup35 unable to propagate [PSI] (Ter-Avanesyan et al. 1994) or induce [PSI] formation by overproduction (Deratch et al. 1996). The dominant “[PSI] no more” PNM2 mutation (Doel et al. 1994) apparently inhibits growth and/or propagation of the prion polymers that contain mutant Sup35 protein (Kochneva-Pervukhova et al. 1998). We have confirmed that the centromeric plasmid bearing the SUP35-PNM2 allele (pFL38-SUP35-PNM2) is a semidominant inhibitor of [PSI] and is unable to maintain the [PSI+] state in the absence of a wild-type SUP35 gene in our strains (P. Bailleul, A. Zink and Y. Chernoff, unpublished data). The two-hybrid experiments (Figure 2A) show that Δ22/69 deletion and PNM2 substitution greatly decrease the ability of the Sup35N domain to interact with the Sla1C. The Δ22/69 deletion also inhibited or greatly decreased Sup35N interactions with Reg1, EF-2 (not shown), and Eno2 (Figure 2A), while the effect of the PNM2 mutation on the interaction between Sup35N and some two-hybrid potentials other than Sla1 (e.g., Eno2) was less evident (Figure 2A). These data suggest that (1) identical or overlapping sequence elements of the Sup35N control prion propagation and interaction with Sla1C and (2) Sup35N-Sla1C interaction is highly specific, since it could be disrupted by just one aa substitution.

**Efficient Sup35N-Sla1C interaction requires Hsp104 chaperone:** An intermediate level of the chaperone protein Hsp104 is required for [PSI] maintenance (Chernoff et al. 1995). We compared efficiencies of the Sup35N-Sla1C two-hybrid interactions in the isogenic Hsp104+ and Hsp104− strains. Our results (Figure 2B) show that efficient Sup35N-Sla1C interaction can be detected only in the presence of Hsp104. Transformation of the hsp104Δ strain with the centromeric plasmid pYS104 bearing the wild-type Hsp104 gene restored the Sup35N-Sla1C interaction (not shown). In the absence of Hsp104, two-hybrid interactions between Sup35N and some other proteins (e.g., Eno2) were not affected as significantly as interactions between Sup35N and Sla1C (Figure 2B). The hsp104Δ had no effect on interactions between the SNF subunits (see material and methods) used as a positive control (not shown), confirming that activation of the gal-inducible promoters does not depend on Hsp104. These data confirm that the hsp104 deletion specifically inhibits both [PSI] propagation and the two-hybrid interaction between the Sup35N and Sla1C domains.

**[PSI] stability is decreased in a [PSI+] sla1Δ strain:** To check whether Sla1 has any effect on [PSI] prion, we have disrupted the SLA1 gene in the [PSI+] strain OT56 (Figure 3). The resulting disruptant remained [PSI−], suggesting that Sla1 protein is dispensable for [PSI] propagation. Moreover, the sla1Δ did not exhibit reproducible effects on either the efficiency of [PSI]-mediated translational readthrough (measured by growth on −Ade medium due to suppression of the ade1-14 mutation) or the distribution of Sup35 protein among soluble and insoluble (polymerized) fractions in [PSI+] cells (not shown). However, [PSI] curing by DMSO or...
than Hsp104 expression. In contrast, no statistically significant differences in [PSI] stability between isogenic Sla1 strains in the [psi−] strain OT60 and GT88 (sla1Δ) [psi−pin], GT17 (SLA1+) and GT83 (sla1Δ). The Sup35 or Sup35N overexpression was achieved by transforming the yeast strains with either plasmid pEMBL-yex-SUP35 or plasmid pEMBL-yex-SUP35ΔBal, respectively. Transformants bearing the control plasmid pEMBL-yex were used as a control. Suppression was detected by growth on −Ade medium after 5 days of incubation. The suppressor effect of excess Sup35 and Sup35N is inhibited in the sla1Δ strain. Color assay on YPD medium confirms the inhibitory effect of sla1Δ on excess Sup35- or Sup35N-mediated suppression (not shown). There was no difference between Sla1 and Sla1 strains in growth on complete medium or on medium selective for the plasmid (not shown). (C) Excess Sup35 (or Sup35N) induced formation of [PSI] in the isogenic Sla1 and Sla1− [psi−PIN+] strains (OT60 and GT88, respectively). The strains were transformed with the plasmids CEN-GAL-SUP35 (Sup35) and pRS316GAL-SUP35N (Sup35N). Sup35 or Sup35N overproduction was induced on galactose medium. After 3 days of incubation on galactose, cells were velvetep replica plated onto −Ade/glucose medium, where the GAL promoter is turned off. Plates were photographed after 3 days of incubation. Growth on −Ade is indicative of [PSI] induction. The Ade+ colonies are further analyzed and confirmed to contain the inheritable GuHCl-curable [PSI] elements. (D) Extra-copy of the SLA1 gene inhibits nonsense suppression induced by excess Sup35 in the [psi−PIN+] strain OT60. Plasmid combinations: control, pRS316GAL + YEp13; Sup35, pRS316GAL + pSTR7; Sup35N, pRS316GAL + pSTR7; Sup35 plus Sup35N. Suppression was detected as growth on −Ade after 5 days of incubation.

by excess Hsp104 was significantly increased in the sla1Δ strain compared to the isogenic SLA1+ strain (Figure 3A). Levels of overproduced Hsp104 were not changed in the sla1Δ strain compared to the isogenic SLA1+ strain (not shown), suggesting that sla1Δ affects [PSI] protection and/or recovery from [PSI] curing agents, rather than Hsp104 expression. In contrast, no statistically significant differences in [PSI] stability between isogenic SLA1+ and sla1Δ strains were observed during growth in liquid YPD medium containing 5 mM GuHCl (not shown). Moreover, DMSO, which cures [PSI] much less efficiently than GuHCl in the SLA1+ strain, becomes more efficient than GuHCl in the sla1Δ strain.

Translation readthrough and [PSI] induction, induced by excess Sup35 or Sup35N, are affected by Sla1 levels: [PSI] formation can be induced de novo in [psi−] strains by overproduction of Sup35 (Cheroff et al. 1993) or Sup35N (Derkatch et al. 1996). This process is affected by the non-Mendelian determinant [PIN] (Derkatch et al. 1997): the Sup35 overproduction causes translational suppression (Cheroff et al. 1992a) and [PSI] induction (Cheroff et al. 1993) only in [psi−PIN+] strains (Derkatch et al. 1997), while the Sup35N overproduction causes suppression (Ter-Avanesyan et al. 1993) and [PSI] induction (Derkatch et al. 1996) in both [psi−PIN+] and [psi−pin−] strains (Derkatch et al. 1997). Efficiency of suppression and frequency of [PSI] induction did usually correlate to each other (Derkatch et al. 1996, 1997). To check whether Sla1 affects initial formation of [PSI] in the [psi−] strains, we disrupted the SLA1 gene in the [psi−PIN+] strain OT60. The isogenic [psi−pin−] sla1Δ derivative was obtained by GuHCl treatment. Our results show that sla1Δ slightly decreases translational suppression by overproduced Sup35 in [psi−PIN+] strains and drastically decreases translational suppression by overproduced Sup35N in

![Graph](image_url)
both [psi\top \cdot PIN\top ] and [psi\top \cdot pin\top ] strains (Figure 3B). Efficiency of [PSI\top ] formation, induced by the transient overexpression of a GAL::SUP35 or GAL::SUP35N construct, is also decreased in the [psi\top \cdot PIN\top ] sla1\top \Delta strain, compared to the isogenic [psi\top \cdot PIN\top ] SLA1\top \Delta strain (Figure 3C). These data suggest that excess Sup35- or Sup35N-mediated translational readthrough and prion formation are facilitated by the Sla1 protein.

We have also observed that suppression of adel-14 by overproduced Sup35 in the [psi\top \cdot PIN\top ] strain is decreased in the presence of extra-copy of the SLA1 gene (Figure 3D). This suggests that intermediate levels of Sla1 protein are required for the maximal efficiency of the extra Sup35-induced translational readthrough.

**Sla1\top \Delta causes sensitivity to translational inhibitors:** Since the Sla1 protein exhibits two-hybrid interaction with a release factor, we asked if Sla1 plays any role in translation. Our data show that sla1\top \Delta strains exhibit increased levels of sensitivity to translational inhibitors such as hygromycin (Figure 4A), paromomycin (Figure 4B), and G418 (not shown) compared to the isogenic SLA1\top \Delta strains. The [PSI\top ] strains are also slightly sensitive to translational inhibitors, and we observed that sla1\top \Delta and [PSI\top ] have additive effects on antibiotic sensitivity (Figure 4). These results suggest a functional role for the Sla1 protein in translation.

**Inhibitory effects of Sla1 on [PSI\top ] strains and some omnipotent suppressor mutants:** We observed that the multicopy plasmid pFL44-SLA1, bearing the SLA1 gene, inhibits growth of the [PSI\top ] strain OT56 but does not inhibit growth of the isogenic [psi\top ] strain OT60 in the conditions that are selective for the plasmid (Figure 5A). The plasmid pFL44-SLA1 is also lost with extremely high frequency in the strain OT56 (but not in the strain OT60) in nonselective conditions, apparently due to a growth disadvantage caused by this plasmid in the presence of [PSI\top ]. This suggests that the overproduction of the Sla1 protein from a multicopy vector is incompatible with [PSI\top ] prion.

To check whether the Sla1 and Sup35 proteins interact functionally in genetic assays, we compared spectra of the spontaneous omnipotent suppressor mutations in the isogenic [psi\top ] SLA1 and [psi\top ] sla1\top \Delta strains (Table 3). In contrast to previous observations by other authors (Holtzman et al. 1993), sla1\top \Delta did not cause significant temperature sensitivity in the strains used in this study. However, the percentage of temperature-sensitive sup35 and sup45 mutants was increased in sla1\top \Delta strains compared to isogenic SLA1\top \Delta strains (Table 3). The differences between the SLA1 and sla1\top \Delta strains were statistically significant, according to the \( \chi^2 \) statistics: 0.01 < \( P(H_0) \) < 0.025. To check whether these mutants remained temperature sensitive after reintroduction of the SLA1 gene, we transformed six temperature-sensitive sup35 mutants, obtained in the sla1\top \Delta background, with the centromeric plasmid YCp-SLA1, bearing the wild-type SLA1 gene. Surprisingly, five of these mutants exhibited severe growth defects in the presence of the SLA1 plasmid, resulting in poor growth on the medium selective for the SLA1 plasmid, even at permissive temperature (Figure 5B), and an extreme plasmid instability in nonselective conditions. Therefore, a specific subset of the sup35 mutant alleles, which are lethal or
TABLE 3  
Spectra of the omnipotent suppressor mutants in the SLA1 and sla1Δ strains

<table>
<thead>
<tr>
<th>Mutants</th>
<th>sup35</th>
<th>sup45</th>
<th>Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ts</td>
<td>non-ts</td>
<td>Σ</td>
</tr>
<tr>
<td>SLA1</td>
<td>1</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>sla1Δ</td>
<td>6</td>
<td>25</td>
<td>31</td>
</tr>
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sublethal in the presence of the Sla1 protein, can be recovered in the sla1Δ background. This result points to the specific genetic interaction between the SUP35 and SLA1 genes.

DISCUSSION

Proteins that interact with the Sup35N domain: It has been shown previously that the N-proximal region of the Sup35 protein (Sup35N) and the highly charged middle portion (Sup35M) are dispensable for cell viability and translational termination function (Ter-Avanesyan et al. 1993). However, the Sup35N domain is required for prion propagation (Doel et al. 1994; Ter-Avanesyan et al. 1994), as well as for overproduction-induced translational readthrough (Ter-Avanesyan et al. 1993) and prion appearance (Derkatch et al. 1996). This piece of the Sup35 protein can therefore be designated as the prion-forming domain. As in the case of the mammalian PrP protein, the normal cellular function of the Sup35 prion-forming domain remains unclear.

Our two-hybrid experiments suggest that the Sup35N domain is involved in a variety of protein-protein interactions, including both translational components (EF-2) and some other proteins, among which two groups were identified. One group includes proteins involved in glucose metabolism, such as Reg1 and Eno2. Further experiments are needed to investigate whether positive results of the two-hybrid assays correspond to any biologically meaningful interactions between these proteins and Sup35N. It is worth noting that the efficiency of translational suppression mediated by [PSI] or some sup35 mutant alleles has previously been shown to depend on the carbon source (Inge-Vechtomov et al. 1988b). Molecular mechanisms of this dependence remain unknown.

Another group of the Sup35N-interacting proteins is represented by the Sla1 protein, whose normal function is to assist in the formation of actin microfilaments constituting the cortical cytoskeletal networks. Overproduction of some cytoskeleton-related proteins has previously been shown to cause severe growth defects in yeast (Liu et al. 1992). The two-hybrid library we used is based on a multicopy plasmid. This may explain why our search failed to uncover cytoskeletal proteins other than Sla1. We knocked out the SLA1 gene and detected numerous effects on [PSI] and sup35. Our data lead to the conclusion that the positive result in the two-hybrid assay reflects an actual biologically meaningful interaction between the Sla1 and Sup35 proteins.

Comparison of the Sla1 and Sup35 structures: Comparison of the Sup35 and Sla1 structures points to the remarkable structural similarity between these two proteins (see Figure 6). Both Sup35 and Sla1 contain (1) termini (Sup35N and Sla1C, respectively), which are rich in Pro, Gly, and Gln and include oligopeptide repeats; (2) highly charged Glu-rich middle portions (Sup35M and Sla1M, respectively); and (3) remaining regions (Sup35C and Sla1N, respectively) that encompass about 2/3 of each protein and contain domains apparently found in other proteins of similar function (GTP-binding domains and aminoacyl-tRNA-binding domains, in the case of Sup35, and SH3 domains, in the case of Sla1). Quite remarkably, the Pro-, Gly-, and Gln-rich Sup35N and Sla1C are the fragments that interact with each other in the two-hybrid assay. It is possible that the presence of the oligopeptide repeats is an indicator of the intermolecular interactions. Mammalian PrP contains oligopeptide repeats similar to those found in Sup35 (Cox 1994; Kushnirov et al. 1995). A number of other cytoskeletal proteins contain Pro-rich repeats.

Figure 6.—Structural and functional organization of the Sup35 and Sla1 proteins. G, GTP-binding domains; A, aa-tRNA-binding domain; SH3, Src homology 3 domains. Regions of oligopeptide repeats and highly charged middle regions are shown. See comments in the text.
among them Paramecium epiplasmins (Coffe et al. 1996). It has been hypothesized that Pro-rich repeats of epiplasmins are responsible for the self-assembly of fibrillar structures.

Parallels between Sla1C-Sup35N interactions and prion propagation: Our data show that the Sup35N-Sla1C two-hybrid interactions are inhibited by Sup35N alterations, which have previously been shown to inhibit propagation of the [PSI] prion. Even the single aa substitution, SUP35-PNM2, greatly decreases efficiency of the Sup35N-Sla1C interaction. The Sup35N-Sla1C two-hybrid interaction also depends on the presence of the chaperone protein Hsp104, which is required for [PSI] propagation. These results confirm that the Sup35N-Sla1C interactions are highly specific and require the same sequence elements and protein helpers that are essential for the propagation of the [PSI+] state.

Role of Sla1 protein in cytoskeletal assembly and prion formation and propagation: The cortical cytoskeleton is a highly dynamic network, which undergoes rapid processes of assembly and disassembly, related to the polarized cell growth, bud formation, cytokinesis, and environmental signals (see Welsh et al. 1994; Ayscough et al. 1997). While Sla1 protein by itself is not required for cell viability and for the cortical cytoskeleton formation, sla1 mutations and deletions become lethal in combination with deletions or mutations of some other genes coding for cytoskeletal assembly proteins, such as Abp1 (Holtzman et al. 1993), Bee1 (Li 1997), and Pan1 (Tang and Cai 1996).

Sla1 is the first cytoskeleton-related protein shown to affect the maintenance of a prion. [PSI] sensitivity to the curing agents, such as DMSO and overproduced Hsp104, is greatly increased in strains lacking the Sla1 protein. We have previously reported that overproduction of the Sup35 protein or, even more efficiently, overproduction of its Sup35N domain causes translational readthrough (Chernoff et al. 1992a; Ter-Avanesyan et al. 1993) and increases spontaneous formation of the [PSI] prion (Chernoff et al. 1993; Derkat et al. 1996). This could be explained as a consequence of aggregation of the overproduced Sup35 protein. Sup35 aggregates can titrate out and inactivate other components of the release factor complex and facilitate nucleation of the new prion “seeds.” Both translational readthrough and [PSI] induction by overproduced Sup35 protein and especially by overproduced Sup35N are greatly decreased in the sla1 deletion strains.

Taken together, our data suggest that while Sla1 protein is not required for prion appearance and maintenance, it is assisting in these processes. Such a role for Sla1 protein in prion maintenance is consistent with the role played by this protein in the assembly of cortical cytoskeleton.

Molecular models of Sla1 effects: Morphological changes observed in sla1 deletion strains (Holtzman et al. 1993) and some patterns of response to the actin inhibitors (Ayscough et al. 1997) suggest that the absence of Sla1 results in the formation of a smaller number of larger filamentous actin structures compared to the wild-type strain. This indicates that the Sla1 protein might assist in the nucleation of new microfilaments. Microfilament nucleation in the growing buds usually does not occur by the simple addition of actin to the “barbed” (growing) ends of the preexisting microfilaments. Rather, new “nuclei” are formed with the participation of proteins other than actin. Experiments on permeabilized cells identify Sla1 as one of the proteins participating in the formation of these new “nuclei” (Li et al. 1995). Therefore, Sla1 is involved in the formation of protein complexes, which are responsible for initiation and growth of polymeric structures. Our data suggest a similar relationship between Sla1 and prion polymers.

Under normal conditions, Sla1 appears to have only a slight or no effect on replication and transmission of preexisting prion polymers. This is in agreement with Sla1’s dispensability for the normal functioning and reproduction of the cytoskeleton in vivo (Holtzman et al. 1993). Apparently, Sla1’s loss could be compensated by other proteins. Sla1 becomes important when normal reproduction of [PSI] aggregates is altered by cellular (overproduced Hsp104) or chemical (DMSO) factors. In this situation, the absence of Sla1 causes a dramatic decrease in [PSI] stability. We propose that Sla1 is taking part in prion “recovery” when [PSI] aggregates are broken down into oligomers or misfolded monomers. Such a recovery could be achieved by generating new “nucleation” sites due to the direct association of the Sla1 and Sup35 molecules.

Interestingly, the sla1 deletion does not seem to significantly affect [PSI] sensitivity to GuHCl. This points out the difference between the mechanisms of [PSI] curing by GuHCl, on one hand, and DMSO and excess Hsp104, on the other hand. Thus, [PSI] curing by GuHCl is not readily explained as a simple consequence of Hsp104 induction by GuHCl, as hypothesized previously (Chernoff et al. 1995). One possibility could be that GuHCl acts by inactivating the chaperone helpers required for [PSI] reproduction, such as Hsp104. Such inactivation could result either from the accumulation of misfolded proteins that aggregate and titrate Hsp104 out, or from direct inactivation of the ATPase activity of Hsp104 by GuHCl, as suggested by in vitro results (Glover and Lindquist 1998). This is in agreement with our observation that both GuHCl treatment and Hsp104 inactivation (but not Hsp104 overproduction) can cure yeast cells of both [PSI] and [PIN] factors. If the Hsp104 requirement for [PSI] propagation is explained by its role in promoting the partitioning and segregation of Sup35N aggregates, as hypothesized recently (Paushkin et al. 1996), then a lack of Hsp104 function would result in the formation of larger polymers, which are unable to segregate and would be lost...
in subsequent cell divisions. Such an effect would less likely be reversed by the nucleating activity of Sla1, which presumably increases formation of the new complexes from monomers and oligomers produced by Hsp104.

Another case in which the presence of Sla1 becomes important is de novo formation of prion aggregates. The most likely explanation of Sla1’s role in [PSI] induction is that Sla1 assists in de novo formation of protein complexes, which serve as new prion nuclei. These complexes also titrate out other components of the translational machinery, resulting in translational read-through.

Sla1 overproduction does have an inhibitory effect on translational suppression by overproduced Sup35. This could be due to the stoichiometry of the interaction between Sup35 and Sla1. In the presence of excess Sla1, a larger number of polymerization nuclei could be formed. Therefore, the average size of aggregates would be decreased, and the possibility for Sup35 to perform its normal function in termination would be increased.

**Functional role of the Sla1-Sup35 interactions:** Our genetic data clearly point to physical and functional interactions between the cytoskeletal assembly protein Sla1 and the translational termination complex in yeast. Interrelationships between the yeast translational termination complex and some cytoskeletal structures were suspected due to the observation that some sup35 and sup45 mutants are sensitive to benomyl, a drug that affects the formation of microtubules (Tikhomirova and Inge-Vechtomov 1996). However, our data provide the first evidence that specifically points to the cortical microfilaments rather than microtubules, since Sla1 is known to be specifically involved in the assembly of microfilaments (Holtzman et al. 1993).

Some components of the translational machinery (Edmonds et al. 1991a), among them EF-1α (Yang et al. 1989) that is partially homologous to the Sup35C (Kushnir et al. 1988), were previously shown to interact with cytoskeletal structures, particularly with actin microfilaments. These interactions may be involved in the intracellular localization of the translational complexes. In Dictostelium, 99% of the soluble (non-ribosome-associated) EF-1α is bound to soluble actin (Edmonds 1993). Actin polymerization releases EF-1α and may therefore activate translation locally. Feedback regulation of the cytoskeletal assembly by variations in translation efficiency could be proposed by a similar mechanism.

Sla1's effects on growth in [PSI+] strains and some sup35 mutant strains (Figure 6) suggest that the normal function of the Sup35 prion-forming domain (Sup35N) could be to interact with multiprotein complexes such as cytoskeletal networks. This interaction is mediated by cytoskeletal assembly proteins such as Sla1 and is responsible for the regulation of the Sup35 translational function through transient inclusion of Sup35 into formations that are not easily accessible for the translational machinery. If Sup35 is already partially inactivated by mutation or prion formation, such an inclusion may cause poor growth or lethality. The sup35 mutations, which increase Sup35 affinity to Sla1, may also have a lethal or sublethal effect in the presence of Sla1. When the Sup35N domain is overproduced and/or its normal targets are missing (e.g., in vitro), Sup35N regions of various molecules begin to interact among themselves, leading to the assembly of prion aggregates. Proteins such as Sla1, which are normally taking part in cytoskeletal formation, are assisting in aggregate assembly as well. Therefore, formation of the [PSI] prion may result as a byproduct of the process, which is normally responsible for interactions between the translational machinery and cytoskeletal networks.

**Similarities between protein-protein interaction patterns of the Sup35N and huntingtin:** Expansions of the N-proximal poly-Gln stretch of huntingtin are associated with Huntington disease and were suggested to promote huntingtin aggregation (Schierzinger et al. 1997). Since formation of the huge huntingtin inclusions does not necessarily correlate with cell death (Saudou et al. 1998), it could be suggested that poly-Gln-expanded huntingtin kills neurons by titrating out essential cellular proteins. Indeed, it has been shown that poly-Gln-expanded huntingtin derivatives possess protein-interacting abilities that are different from those of the normal huntingtin. For instance, poly-Gln expansion promotes interaction between huntingtin and the SH3-containing protein SH3GL3 (Sittler et al. 1998) and inhibits the interaction between huntingtin and another protein, HIP1 (Kalchman et al. 1997). SH3 domains are characteristic of some cytoskeletal assembly proteins, including Sla1, while HIP1 is a human homologue of the yeast Sla2, a cytoskeletal assembly protein identified in the same genetic screen as Sla1 (Holtzman et al. 1993). This suggests that poly-Gln stretches of huntingtin may be involved in interactions with cytoskeletal structures. Indeed, we observed that expansion of the poly-Gln stretch enables the N-proximal huntingtin fragment to interact with the yeast Sla1C protein in two-hybrid assays (Figure 1C). Moreover, the poly-Gln-expanded huntingtin is also interacting with other proteins (EF-2, Reg1, and Eno2) that recognize Sup35N regions of Sup35N, which increase Sup35 affinity to Sla1, may also have a lethal or sublethal effect in the presence of Sla1. When the Sup35N domain is overproduced and/or its normal targets are missing (e.g., in vitro), Sup35N regions of various molecules begin to interact among themselves, leading to the assembly of prion aggregates. Proteins such as Sla1, which are normally taking part in cytoskeletal formation, are assisting in aggregate assembly as well. Therefore, formation of the [PSI] prion may result as a byproduct of the process, which is normally responsible for interactions between the translational machinery and cytoskeletal networks.

**Comparisons to other prions and polymeric proteins:** The mammalian PrP is a surface protein associated with...
cellular membrane structures. It is possible that PrPSc formation may result as a by-product of the molecular process, which is normally responsible for interactions between PrP and its partners. It is also worth noting that PrPSc plaques, as well as some other amyloid deposits and neural inclusions, involve cytoskeletal proteins (Guiray et al. 1989; Liber ski 1994). In general, one could propose that proteins involved in interactions with highly ordered cellular and extracellular structures, such as cytoskeletal formations, may possess a potential to form alternative types of structures, which become reproducible in a template-like fashion. Prions represent just one example of such “structural coding.” Another well-known example is “cortical memory,” or “cytotaxis” in Paramecium (Beisson and Sonneborn 1965). Further experiments are required to determine how widespread such systems of structural coding really are.

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