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RESEARCH ARTICLE

[*NSI*$^+$]: a novel non-Mendelian nonsense suppressor determinant in *Saccharomyces cerevisiae*

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Abstract Non-Mendelian determinants that control heritable traits in yeast are subdivided into two major groups—one that includes DNA- or RNA-based elements and another that comprises protein-based factors that are analogous to mammalian prion. All yeast non-Mendelian determinants show dominant inheritance, and some of them demonstrate cytoplasmic infectivity. Only prions, however, harbor-specific features, such as high frequency of induction following overproduction of prion-encoding protein, loss of the protein's normal function, and reversible curability. Here, we describe a novel nonchromosomal determinant that, in addition to [*PSI*$^+$] and [*ISP*$^+$], is involved in epigenetic control of nonsense suppression. This determinant, which we have designated [*NSI*$^+$], causes nonsense suppression in the strains bearing the N-terminal-deleted or -modified *SUP35* gene, but has no manifestation in the strains with the intact copy of *SUP35*. [*NSI*$^+$] shows dominant non-Mendelian inheritance, reversible curability and may be transmitted by cytoduction, albeit with low frequency. Similar to yeast prions, this determinant can be cured by deletion or mutational inactivation of Hsp104. We have shown that [*NSI*$^+$] does not correspond to the already identified yeast prions. Based on the data obtained, we hypothesize that [*NSI*$^+$] is a novel prion factor involved in epigenetic control of nonsense suppression.

Keywords Yeast · Non-Mendelian determinant · Prion · Nonsense suppression · Sup35

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Introduction

Yeast non-Mendelian genetic determinants epigenetically modulate fundamental biological processes. The inheritance properties of the *petite*, *killer*, 20S RNA factors and the 2μ plasmid are determined by nucleic acids that can readily be detected in yeast cells (Cox 1994). Other determinants, however, do not contain DNA or RNA components. In 1994, Wickner (1994) proposed that yeast non-Mendelian elements [*URE3*] and [*PSI*$^+$] are similar in their fundamental properties to mammalian prion. A set of yeast proteins capable of generating self-perpetuating amyloid-like aggregates has been identified to date (Wickner 1994; Derkatch et al. 1997; Du et al. 2008; Alberti et al. 2009; Nemecek et al. 2009; Patel et al. 2009). The inheritance and infectious properties of some protein-based yeast determinants, such as [β] and [*GAR*$^+$], are not associated with amyloidogenesis (Roberts and

Wickner 2003; Brown and Lindquist 2009). Amyloid-based yeast prions, unlike mammalian PrP, contain Q/N-rich tracts depleted of hydrophobic and charged residues. This composition is important for amyloid polymerization and prion propagation (Ross et al. 2005). Propagation of these prions is regulated by chaperone proteins—in particular, Hsp104. Using free energy from ATP hydrolysis, Hsp104 splits up large prion polymers into small oligomers that initiate new prion conversion cycles (Paushkin et al. 1996; Jung and Masison 2001; Chernoff et al. 1995). When the *HSP104* gene is deleted or its ATPase activity is abolished, prion aggregates cannot dissemble into small oligomers and fail to enter daughter cells (Kushnirov and Ter-Avanesyan 1998). Guanidine hydrochloride (GuHCl) eliminates yeast prions by inactivating Hsp104 (Ferreira et al. 2001). Thus, chaperone machinery is required for prion propagation, but it does not affect other non-Mendelian determinants.

An in silico analysis of the yeast proteome revealed approximately 170 Q/N-rich proteins that potentially might be the prions (Michelitsch and Weissman 2000; Harrison and Gerstein 2003). In a systematic survey, Lindquist and colleagues identified 19 protein domains that possess some amyloid or prion characteristics (Alberti et al. 2009). Taken together, these data suggest that the real number of prion proteins may be much more than we knew so far.

Prion formation usually results in the partial loss of a protein's normal function, allowing it to be monitored in specifically designed phenotypic assays. For instance, [*PSI*$^+$], the prion isoform of Sup35p, was originally described as a non-Mendelian factor that controlled omnipotent nonsense suppression (McCready et al. 1977). Sup35p, a translation termination factor (Stansfield et al. 1995; Zhouravleva et al. 1995), contains three domains: a C-terminal domain that is essential for translation termination and cell viability, an M (middle) domain whose function is unknown, and an N-terminal domain that is dispensable for translation termination and viability but is essential for [*PSI*$^+$] formation (Ter-Avanesyan et al. 1993). The N-terminal prion-forming domain of Sup35 mediates mRNA decay through the regulation of deadenylation (Hoshino et al. 1999; Hosoda et al. 2003).

In this paper, we describe a new nonchromosomal yeast determinant that, like [*PSI*$^+$], regulates nonsense suppression. This determinant, which we have designated [*NSI*$^+$] (nonsense suppression inducer), causes the suppressor phenotype in strains that contain the *SUP35* gene in which the N-prion-forming region has been deleted or modified. The expression of full-length *SUP35* masks, but does not eliminate the [*NSI*$^+$]. Like known yeast prions, [*NSI*$^+$] shows reversible curability, is eliminated by Hsp104 deletion or inactivation, and demonstrates non-Mendelian inheritance and cytoplasmic infectivity.

Materials and methods

Plasmids

All plasmids used in this work are shuttle vectors that replicate in *S. cerevisiae* and *E. coli*. The vector pRS315 was described previously (Christianson et al. 1992). The plasmid P316-Sp-SUP35 bears the modified version of the *SUP35* gene, which contains additional restriction sites and is expressed from its native promoter (DePace et al. 1998). The plasmid pmCUPNMsGFP encodes the Sup35NM-GFP-fused protein (Serio et al. 1999). The plasmids pFL38-SUP35-Δ3ATG that encodes a Sup35C domain and pFL38-SUP35P that contains the *Pichia methanolica SUP35* gene (*SUP35 P.m.*) were kindly provided by S. Zadorsky (Derkatch et al. 2000). The single-copy plasmid pRS315-SUP35MC (unpublished) was kindly provided by Y. Chernoff. This plasmid was constructed by inserting the PCR-generated 0.4 kb *Bam*HI–*Eco*RI fragment, containing the *SUP35M* sequence, into P316-Sp-SUP35 digested with *Bam*HI and *Eco*RI. As a result, the *SUP35NM* region that encodes both N-terminal (N) and middle (M) domains was replaced by *SUP35M*. In order to construct the plasmid pU-SUP35MC which bears the *URE3* marker, the *Xho*I–*Sac*I fragment of the pRS315-SUP35MC containing the P_{SUP35}-*SUP35MC* cassette was inserted into the vector pRS316. The pmCUP1-SUP35MC plasmid was constructed as follows: The *Xho*I–*Bam*HI fragment containing the copper-inducible *CUP1* promoter (P_{CUP1}) from the vector pmCUP1 (Serio et al. 1999) was inserted into pRS425 plasmid (Labbe-Bois 1990). The *Bam*HI–*Sac*I fragment that encodes the *SUP35MC* from the pRS315-SUP35MC plasmid was inserted downstream of the P_{CUP1}. The pYCH-U2 is the single-copy plasmid containing *SUP35* and *URA3* genes (Derkatch et al. 1997). The pSTR7 is the multicopy plasmid containing *SUP35* and *LEU2* genes (Chernoff et al. 1993). pU-Aβ-SUP35MC (Tsaponina et al. 2005) and pL-Aβ-SUP35MC are the single-copy plasmids, bearing the sequence encoding a human amyloid-beta peptide 1–40 amino acids (hereinafter referred to as Aβ), fused with the *SUP35MC* fragment under the P_{CUP1}. In order to construct the plasmid pL-Aβ-SUP35MC which bears the *LEU2* marker, the 3.3 kb *Xho*I–*Sac*I fragment of the pU-Aβ-SUP35MC containing the P_{CUP1}-*Aβ-SUP35MC* cassette was inserted into the vector pFL36 (Bonneaud et al. 1991). The YEpHO plasmid was described earlier (Jensen et al. 1983). The pLH105 multicopy plasmid contains the *HSP104* gene under the control of the *GPD* promoter (Vogel et al. 1995). The pRS424-GPD-HSP104-KT plasmid bearing the mutant *HSP104-KT-218, 620* gene under the control of the *GPD* promoter was described earlier (Rubel et al. 2008). The pYSL5 plasmid contains *LEU2* gene flanked by the 5′ and 3′ non-coding sequences of *HSP104* gene (Sanchez and Lindquist 1990). To provide the overexpression of *SUP35*,

Table 1 Strains of *S. cerevisiae* obtained in this work

Strain	Genotype
D931	MATα/MATa *SUP35/sup35Δ::HIS3 his3/his3 ade1-14/ade1-14 trp1-298/trp1 lys2/lys2 ura3/ura3 leu2/leu2* [pU-Aβ-Sup35MC] [psi^-][PIN^+]
1-D931	*MAT*a *sup35Δ::HIS3 ade1-14 his3 leu2 lys2 ura3 trp1-289* [pU-Aβ-Sup35MC] [PIN^+]
1-1-D931	[NSI^+] derivative of 1-D931
1-1-1-D931	[nsi^-][pin^-] derivative of 1-1-D931
2-1-1-D931	Derivative of 1-1-D931, bearing pRS315-SUP35MC instead pU-Aβ-Sup35MC
4-1-1-D931	Derivative of 1-1-D931, bearing pL-Aβ-Sup35MC instead pU-Aβ-Sup35MC
1-4-1-1-D931	[nsi^-][pin^-] derivative of 4-1-1-D931
2-4-1-1-D931	Derivative of 4-1-1—D931, bearing pU-Sup35MC instead pL-Aβ-Sup35MC
1-2-1-1-D931	[nsi^-][pin^-] derivative of 2-1-1-D931
2-2-1-1-D931	*MAT*α derivative of 1-2-1-1-D931, bearing pU-Sup35MC instead pRS315-SUP35MC
2-1-1-1-D931	*MAT*α derivative of 1-1-1-D931, bearing pL-Aβ-Sup35MC instead pU-Aβ-Sup35MC
3-1-1-D931	*hsp104Δ::LEU2* derivative of 1-1-D931
2-4-1-1-D931	MATa *sup35Δ::HIS3 ade1-14 his3 leu2 lys2 ura3 trp1-289* [pL-Aβ-Sup35MC] [nsi^-][PIN^+]
6-1-1-D931	MATα *sup35Δ::HIS3 ade1-14 his3 leu2 lys2 ura3 trp1-289* [pU-Aβ-Sup35MC] [NSI^+][PIN^+]
D932	MATα/MATa *SUP35/sup35Δ::HIS3 ADE1/ade1-14 ADE2/ade2-28 HIS3/his3 LEU2/leu2 LYS2/lys2 LYS9/lys 9-21 ura3/ura3-525 trp1/trp1-289 CYH/cyh^R KAR1/kar1-1*[pL-Aβ-Sup35MC][nsi^-][pin^-]
1-D932	MATα *sup35Δ::HIS3 ade1-14 his3 leu2 ura3 trp1-289* cyh^R *kar1-1* [rho^0][pL-Aβ-Sup35MC] [nsi^-][pin^-]
2-1-D932	Derivative of 1-D932, bearing pU-SUP35MC instead pL-Aβ-Sup35MC
D933	MATα/MATa *sup35Δ::HIS3/sup35Δ::HIS3 his3/his3 ade1-14/ade1-14 trp1-298/trp1-289 lys2/lys2 ura3/ura3 leu2/leu2* [pU-Ab-Sup35MC] [pL-Aβ-Sup35MC] [nsi^-][PIN^+]
1-D933	MATα *sup35Δ::HIS3 ade1-14 his3 leu2 lys2 ura3 trp1-289* [pU-Aβ-Sup35MC] [nsi^-][PIN^+]
D934	MATα/MATa *sup35Δ::HIS3/sup35Δ::SUP35P_LEU2 his3 Δ/his3 ade1-14/ade1-14 trp1-298/trp1 LYS2/lys2 ura3/ura3-52 leu2/leu2-3,112* [pU-Ab-Sup35MC] [NSI^+][PIN^+]
1-D934	MATa sup35Δ::SUP35P_LEU2 *ade1-14 his3 leu2 lys2 ura3 trp1* [NSI^+][PIN^+]
2-D934	MATα sup35Δ::SUP35P_LEU2 *ade1-14 his3 leu2 lys2 ura3 trp1* [NSI^+][PIN^+]
D935	MATα/MATa *sup35Δ::SUP35P_LEU2/sup35Δ::SUP35P LEU2 his3/his3 ade1-14/ade1-14 trp1/trp1 LYS2/lys2 ura3/ura3-52 leu2/leu2-3,112* [NSI^+][PIN^+]
D936	MATα/MATa *sup35Δ::SUP35P_LEU2/sup35Δ::SUP35P LEU2 his3/his3 ade1-14/ade1-14 trp1/trp1 LYS2/lys2 ura3/ura3-52 leu2/leu2-3,112* [nsi^-][pin^-]

RNQ1, *URE2*, *SWI1*, *CYC8*, *MCA1*, *MOT3* and *NEW1* genes, the multicopy plasmids from the Yeast Genomic Tiling Collection (Open Biosystems, USA) were used.

Yeast strains

The GT109 MATα/MATa *SUP35/sup35Δ:HIS3 his3/his3 ade1-14/ade1-14 trp1-289/trp1 lys2/lys2 ura3/ura3 leu2/leu2* isogenic diploid strain and its GT111 [psi^-][PIN^+] derivative were described previously (Chernoff et al. 2000). The derivatives of the strains BY4742 (*MAT*α *his3Δ1 leu2Δ lys2Δ ura3*[psi^-][PIN^+]) from BY deletion collection (Invitrogen, USA) contain the deletions of *RNQ1*, *URE2*, *CYC8*, *MCA1*, *MOT3* and *NEW1* genes marked by the *KanMX4* gene. The 7B-D901 (*MAT*a *ade2-28 his3 LEU2 lys 9-21 ura3-525 trp1 cyh^R kar1-1* [pin^-]) strain was kindly provided by J. Sopova. The 1P-74-D694 (MATa sup35Δ::SUP35P_LEU2 ade1-14 his3Δ ura3-52 leu2-3,112 trp1) strain bears the cassette that contains *SUP35 P.m.* and *LEU2* genes integrated into the chromosome instead of *S. cerevisiae SUP35* (Derkatch et al. 2000). Strains obtained in this study are listed in Table 1. The D931 diploid strain was obtained by transformation of the GT111 strain with the pU-Aβ-SUP35MC plasmid. The 1-D931 haploid strain contains the *sup35Δ::HIS3* deletion and the plasmid-borne *Aβ-SUP35MC* construction. It was obtained by sporulation and dissection of the D931 strain. The 1-1-D931 strain is [NSI^+] derivative of the 1-D931 strain. The 4-1-1-D931 strain is a derivative of the 1-1-D931 strain that bears the pL-Aβ-SUP35MC plasmid instead of pU-Aβ-SUP35MC. The [nsi^-][pin^-] 1-1-1-D931 and 1-4-1-1-D931 strains are the derivatives of the 1-1-D931 and 4-1-1-D931 strains, respectively. They were obtained after three consecutive passages on the YPD medium containing both 150 μM $CuSO_4$ and 5 mM GuHCl. The 2-1-1-D931 haploid strain was created by transformation of the strain 1-1-D931 with the pRS315-SUP35MC plasmid followed by elimination of the pU-Aβ-SUP35MC plasmid.

The 1-2-1-1-D931 strain is a derivative of strain 2-1-1-D931 obtained by GuHCl treatment. The 2-2-1-1-D931 strain was obtained as follows: the 1-2-1-1-D931 strain was transformed with the pU-SUP35MC plasmid followed by elimination of the pRS315-SUP35MC plasmid. After that the mating type was switched from *MATa* to *MATα* by the use of the YEpHO plasmid. The 2-1-1-1-D931 strain was obtained as follows: the 1-1-1-D931 strain was transformed with the pL-A*β*-SUP35MC plasmid followed by elimination of the pU-A*β*-SUP35MC plasmid. After that the mating type was switched from *MATa* to *MATα* by the use of the YEpHO plasmid. To obtain the 6-1-1-D931 strain, the mating type of 1-1-D931 strain was switched from *MATa* to *MATα* by the use of the YEpHO plasmid. The 3-1-1-D931 strain is a derivative of the 1-1-D931 strain, which contains the deletion of chromosomal copy of *HSP104*. The 1-D932 cytoduction recipient strain was constructed by mating the 2-1-1-1-D931 [*nsi*$^-$] strain to the 7B-D901 strain followed by sporulation and dissection of the resulting D932 diploid. To obtain 1-D933 strain, the 1-D931 and 2-1-1-1-D931 haploid strains were mated than sporulated. Resulting D933 diploid strain was dissected. The [*psi*$^-$][*PIN*$^+$] haploid segregants that bear the plasmid with *Aβ-SUP35MC* hybrid gene as well as *ade1-14*$_{UGA}$ and *trp1-289*$_{UAG}$ nonsense mutations were selected. The 1-D934 and 2-D934 haploid [*NSI*$^+$][*PIN*$^+$] strains were constructed by mating the 1P-74-D694 strain to the 6-1-1-D931 strain followed by sporulation and dissection of the resulting diploid D934. The D935 [*NSI*$^+$] and D936 [*nsi*$^-$] strains were obtained as described in "Results".

Genetic and microbiological techniques

Standard yeast genetic techniques, media and cultivation conditions were used (Kaiser et al. 1994). Yeast cultures were grown at 30°C. The presence of [*NSI*$^+$] factor was monitored by suppression of the *ade1-14*$_{UGA}$ and *trp1-289*$_{UAG}$ mutations that results in growth on the synthetic medium lacked adenine or tryptophan (Chernoff et al. 2002). Copper sulfate ($CuSO_4$) was added to synthetic and YPD media at various concentrations (as indicated) in order to induce expression of the genes under the P_{CUP1} promoter. To eliminate [*NSI*$^+$] factor, yeast cultures were grown for three consecutive passages on the solid YPD medium with 150 μM of $CuSO_4$ in the presence of 5 mM GuHCl. For cytoduction experiments, the donor [*NSI*$^+$] strain was mated to the karyogamy-defective *kar1* (Conde and Fink 1976) 1-D932 recipient strain on the YPD medium. Cell mixtures were incubated overnight and then replica plated to the synthetic medium, which contained glycerol as the sole carbon source and cycloheximide (5 mg/l). This medium selects for cytoductants that are cycloheximide-resistant, as they originate from the recipient strain, but can grow on glycerol, since they received mitochondria from the donor strain. The [*NSI*$^+$] cytoductants were identified as described in "Results". The protein transformation assay was performed as described previously (Tanaka and Weissman 2006; Patel and Liebman 2007). To introduce [*PIN*$^+$] factor into the [*nsi*$^-$][*pin*$^-$] strain, the MATa 1-1-4-1-1-D931 [*nsi*$^-$][*pin*$^-$] spheroplasts were transformed with the protein extract isolated from the BY4247 MATα [*PIN*$^+$] strain and with the pmCUPNMsGFP plasmid encoding the Sup35NM-GFP protein. To introduce the [*NSI*$^+$] determinant into the [*nsi*$^-$] strain, the MATα 1-D933 [*nsi*$^-$][*PIN*$^+$] spheroplasts were transformed with protein extract isolated from the MATa 4-1-1-D931 [*NSI*$^+$] strain and with the pRS315 vector. The transformants were selected on –Leu–Ura medium with 1 M sorbitol, tested of mating type, and resulting clones were analyzed as described in "Results". In control experiment, the 1-D933 strain was transformed with protein extract isolated from the 1-4-1-1-D931 [*nsi*$^-$] strain and with the pRS315 vector. To analyze the [*NSI*$^+$] spontaneous induction, we performed the fluctuation test as described previously (Volkov et al. 2002). To perform this experiment, three different dilutions from 10^4 to 10^6 cells of 1-4-1-1-D931 [*nsi*$^-$][*pin*$^-$] and 2-4-1-1-D931 [*nsi*$^-$][*PIN*$^+$] strains were used, and each particular dilution was repeated twice. Cells were grown on –Ade + 150 μM $CuSO_4$ medium for 5 days after that the de novo appeared Ade$^+$ clones were streaked for single colonies. To estimate mitotic stability and GuHCl curability, Ade$^+$ clones were passed for three times on YPD + 150 μM $CuSO_4$ and on the same medium with an addition of 5 mM GuHCl. Next, these clones were streaked for the single colonies, selected on YPD + 150 μM $CuSO_4$ and replica plated on –Ade + 150 μM $CuSO_4$ medium.

DNA and protein analysis

Standard procedures were used for DNA isolation and plasmid construction (Sambrook et al. 1989). To obtain the *HSP104* deletion, the pYSL5 plasmid was digested with restriction enzymes *Pvu*I and *Hin*dIII. The *Pvu*I–*Hin*dIII fragment includes the *LEU2* gene flanking by 5′ and 3′ non-coding sequences of *HSP104*. The 1-1-D931 [*NSI*$^+$] haploid strain was transformed with the restriction mix followed by selection of the *hsp104Δ::LEU2* cells on –Leu medium. The deletion of *HSP104* was proved by the use of the *HSP104*-specific primers:

F: 5′-AATTGGTGAGCCAGGTATCGGTAAG;
R: 5′-GACTCGAGCTCTTAATCTAGGTCATCATCAA TTTC.

The preparation of the cell lysates and differential centrifugation was performed as described (Patino et al. 1996). Protein extracts were run on SDS–PAGE gel and reacted to

4G8 Aβ-specific mouse monoclonal antibodies (Sigma) or Sup35-specific rabbit polyclonal antibodies kindly provided by S. Chabelskaya (Chabelskaya et al. 2004). Reactions with the secondary anti-mouse and anti-rabbit antibodies as well as chemiluminescent detection were performed by the use of the ECL detection kit from General Electric (USA). Bradford technique and Coomassie staining were used as a loading control. The intensity of bands on immunoblot was determined by the densitometry using Image J 1.37a software.

Results

Yeast strains lacking the Sup35N domain show GuHCl-curable nonsense suppression

The [*NSI*$^+$] determinant has been originally found in the 1-1-D931 derivative of the haploid yeast strain 1-D931, which harbored the plasmid pU-Aβ-Sup35MC and a deletion of the *SUP35* chromosomal copy (see "Materials and methods"). The chimeric gene *Aβ-SUP35MC* is under the control of the P_{CUP1} and contains a sequence that encodes human Aβ peptide (40 aa), fused in frame to the coding sequence of the *SUP35MC* fragment. This strain, which contained the nonsense alleles $ade1\text{-}14_{UGA}$ and $trp1\text{-}289_{UAG}$, grew on synthetic medium that lacked adenine and tryptophan (Fig. 1a). This indicates that the background level of Aβ-Sup35MC expression from the P_{CUP1} promoter in the absence of extra-copper is insufficient to fully compensate for the translational function of the Sup35, leading to termination defect and readthrough of $ade1\text{-}14_{UGA}$ and $trp1\text{-}289_{UAG}$. An increase in $CuSO_4$ concentration was accompanied by decreased growth on –Ade and –Trp media. We confirmed that Aβ-Sup35MC levels rose gradually with increases in $CuSO_4$ concentration (Fig. 1a). In the presence of 150 μM $CuSO_4$, nonsense suppression was not detected indicating that the complete restoration of termination efficiency occurred, when concentration of chimeric protein increased. However, we selected one clone that grew on –Ade and –Trp media in the presence of 150 μM $CuSO_4$ and streaked it to obtain the single colonies. All colonies retained the ability to grow on –Ade and –Trp media with 150 μM $CuSO_4$ after 2 and 3 days of incubation, respectively (Fig. 1b). The level of Aβ-Sup35MC production in the culture demonstrating the suppressor phenotype was the same as in the original strain (Fig. 1b). As a result of three consecutive passages (20–30 generations) on YPD medium that contained 5 mM GuHCl, the nonsense suppression was eliminated in 123 of 127 clones (97%; Fig. 1b). These data show that GuHCl cures the suppressor determinant designed [*NSI*$^+$]. Strains that lacked [*NSI*$^+$] were referred to as [*nsi*$^-$].

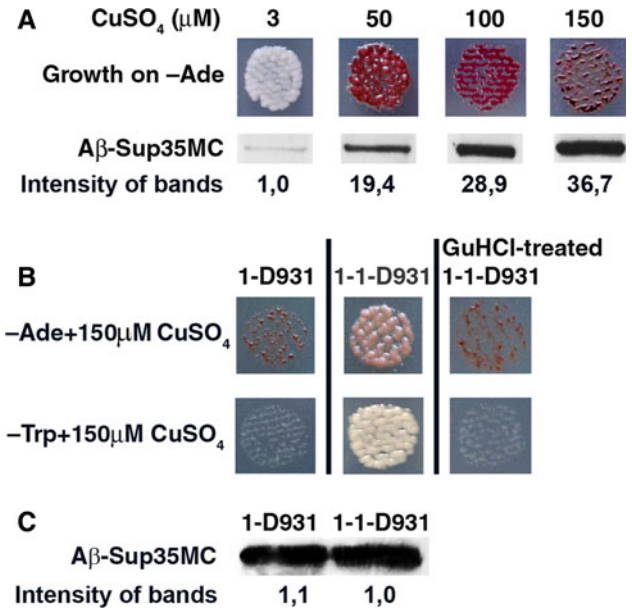

Fig. 1 Nonsense suppression in *sup35Δ::HIS3* [pU-Aβ-Sup35M] strains. **a** Growth of the 1-D931 strain on –Ade medium with various concentrations of $CuSO_4$. Pictures of the plates were taken after 3 days of incubation at 30°C. Proteins were extracted from yeast cultures that were grown on YPD medium in the presence of 3, 50, 100, and 150 μM $CuSO_4$ as shown. Equal amounts of total protein were loaded per sample. Proteins were incubated with the Aβ-specific antibody as described in "Materials and methods". Relative band intensities were determined by densitometry using Image J 1.37a. **b** Growth of the 1-D931 strain and its Ade$^+$Trp$^+$ derivative (1-1-D931) on –Ade + 150 μM $CuSO_4$ and –Trp + 150 μM $CuSO_4$ media before and after GuHCl treatment. **c** The level of Aβ-Sup35MC production in 1-D931 and 1-1-D931 strains is shown. Equal amounts of total protein from 1-D931 and 1-1-D931 strains were loaded per sample

Recently, it was shown that chimeric protein bearing Aβ(42 aa) sequence fused in frame with Sup35MC forms the small oligomers in yeast cytoplasm (Bagriantsev and Liebman 2006). One cannot exclude the possibility that the nonsense suppression in the [*NSI*$^+$] strain results from the Aβ-Sup35MC prion-like conversion. To test this hypothesis, we replaced the pU-Aβ-SUP35MC plasmid with a centromeric plasmid bearing the *SUP35MC* fragment under the *SUP35* promoter. The resulting strain, 2-1-1-D931, which expressed the *SUP35MC* sequence, grew on –Ade after 5–6 days of incubation independently of $CuSO_4$ concentration (Fig. 2a). Notably, the GuHCl-treated 2-1-1-D931 cells failed to grow on –Ade and –Trp media (Fig. 2a). GuHCl-curable suppression was also detected when an Aβ-Sup35MC-encoding plasmid was replaced with the centromeric plasmid pFL38-SUP35Δ3ATG, which contained the *SUP35C* sequence under the control of the *SUP35* promoter (not shown). Thus, the maintenance of [*NSI*$^+$] does not require the presence of Aβ, Sup35N, or Sup35NM sequence. This result confirms that [*NSI*$^+$] is unrelated to Aβ-Sup35MC or Sup35 prion-like conversion.

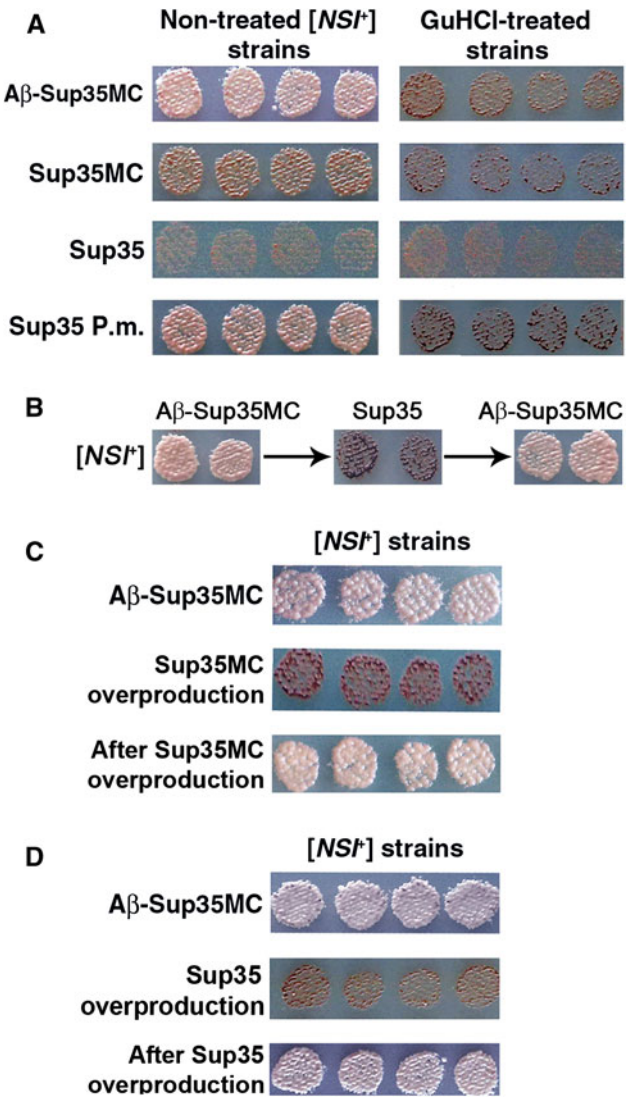

Fig. 2 Effects of Sup35 S.c., Sup35MC S.c., and Sup35 P.m. production on [*NSI*$^+$] manifestation. **a** [*NSI*$^+$] cells bearing the pL-A*β*-SUP35MC plasmid were transformed with the pYCH-U2, pU-SUP35MC and pFL38-SUP35P centromeric plasmids encoding Sup35 S.c., Sup35MC S.c., and Sup35 P.m., respectively, followed by elimination of the plasmid encoding *Aβ-SUP35MC*. The resulting cells were treated with 5 mM GuHCl on YPD medium. The yeast cells in each step of the experiment were replica-plated on –Ade medium. Pictures of plates were taken after 5 days of incubation at 30°C. **b** [*NSI*$^+$] cells bearing the pL-A*β*-SUP35MC plasmid were transformed with the pYCH-U2 plasmid encoding full-length Sup35 S.c. followed by elimination of the plasmid encoding A*β*-Sup35MC. Such shuffle results in elimination of nonsense suppression. At the next step, the plasmid bearing *SUP35 S.c.* was substituted again with A*β*-Sup35MC-encoding plasmid. Resulting derivatives demonstrate the suppressor phenotype. **c**, **d** The 1-1-D931 [*NSI*$^+$] strain was transformed with the pmCUP1-SUP35MC and pSTR7 plasmids for Sup35MC S.c. and Sup35 S.c. overproduction, respectively, and with the pRS315 empty vector. The transformants were selected on –Leu + 150 μM $CuSO_4$ medium and replica-plated on –Ade–Leu + 150 μM $CuSO_4$. Next, the pmCUP1-SUP35MC and pSTR7 plasmids were eliminated. Cells that lost the plasmids for Sup35MC S.c. and Sup35 S.c. overproduction were replica-plated on –Ade + 150 μM $CuSO_4$ medium. Pictures of plates were taken after 3 days of incubation at 30°C

We also transformed the [*NSI*$^+$] strain with the pYCH-U2 plasmid, which encoded the full-length *S. cerevisiae SUP35* (*SUP35 S.c.*), and with the pFL38-SUP35P plasmid, bearing the *P. methanolica SUP35* gene (*SUP35 P.m.*). The cells that expressed *SUP35 P.m.* demonstrated the GuCl-curable suppressor phenotype (Fig. 2a). It is known that Sup35 P.m. is insufficient to fully compensate for the translational function of the Sup35 S.c. (Chernoff et al. 2000), but the [*NSI*$^+$] cells that produce Sup35 P.m. grow on –Ade medium after 2 days of incubation, whereas their GuHCl-treated derivatives start to grow on –Ade medium only after 6–7 days of incubation. Surprisingly, cells that expressed *SUP35 S.c.* did not grow on –Ade medium (Fig. 2a). It should be noted that the *SUP35N* region of *P. methanolica* is highly divergent from that of *S. cerevisiae* (Kushnirov et al. 1990).

To analyze whether the presence of *SUP35N S.c.* is essential for the maintenance of the [*NSI*$^+$], the plasmid bearing *SUP35 S.c.* was substituted again with A*β*-Sup35MC-encoding plasmid. The resulting cells demonstrate the suppressor phenotype (Fig. 2b). Thus, *SUP35N* expression masks [*NSI*$^+$] manifestation, but is not essential for the maintenance of this determinant. Taken together, these data show that [*NSI*$^+$] causes the nonsense suppression only in strains in which the N-terminal domain of Sup35 has been deleted or modified. Overexpression of full-length *SUP35*, as well as *SUP35MC*, masked the nonsense suppressor phenotype; however, the suppression was restored when the plasmids for overexpression of *SUP35* or *SUP35MC* were eliminated (Fig. 2b, c).

To determine whether [*NSI*$^+$] induces the aggregation of the N-terminally deleted or substituted Sup35p, we compared the levels of A*β*-Sup35MC and Sup35MC aggregation in [*NSI*$^+$] and [*nsi*$^-$] cells. Proteins were extracted from [*NSI*$^+$] and [*nsi*$^-$] strains and fractionated into soluble (S) and insoluble (I) fractions by centrifugation at 12,000*g*, as described (Patino et al. 1996; Newnam et al. 1999). Protein extracts from the GT109 [*PSI*$^+$] and GT113 [*psi*$^-$] strains were used as controls. In the [*NSI*$^+$] and [*nsi*$^-$] strains, A*β*-Sup35MC and Sup35MC were detected mostly in the soluble fraction (Fig. 3). A similar ratio of soluble to insoluble Sup35 was observed in the control [*psi*$^-$] strain. These results show that the [*NSI*$^+$] determinant does not affect A*β*-Sup35MC or Sup35MC aggregation.

GuHCl cures yeast prions by inactivating the Hsp104 activity (Ferreira et al. 2001). To determine the effects of this chaperone in our system, the *hsp104Δ::LEU2* derivative of the 1-1-D931 [*NSI*$^+$] strain was obtained (see "Materials and methods"). All of the clones in which the deletion of *HSP104* was confirmed failed to grow on –Ade and –Trp medium that contained $CuSO_4$ (Fig. 4). We also analyzed the effect of the mutational inactivation of Hsp104 on [*NSI*$^+$]. The [*NSI*$^+$] strains 1-1-D931 and 2-1-1-D931

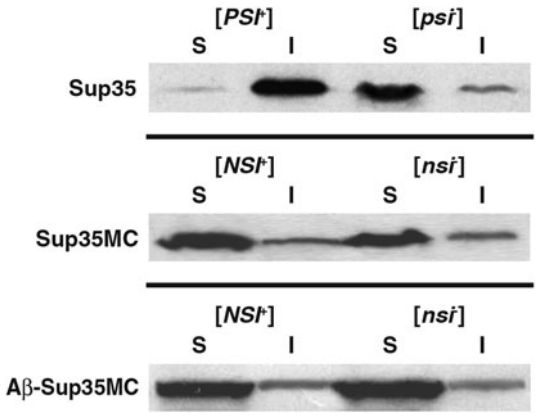

Fig. 3 Analysis of Sup35MC and Aβ-Sup35MC aggregation in [NSI^+] and [nsi^-] strains. Proteins were isolated from the 2-1-1-D931 and 1-1-D931 [NSI^+] strains and its GuHCl-treated [nsi^-] derivatives. The 2-1-1-D931 and 1-1-D931 strains produced Sup35MC and Aβ-Sup35MC, respectively. Protein extracts from GT109 [PSI^+] and GT111 [psi^-] strains were used for control. Cell lysates were fractionated into soluble (*S*) and insoluble (*I*) fractions by centrifugation at 12,000*g*, as described (Newnam et al. 1999), separated on an SDS–PAGE gel, transferred onto a nitrocellulose membrane, and incubated with Sup35-specific antibody. Bradford technique and Coomassie staining were used as a loading control

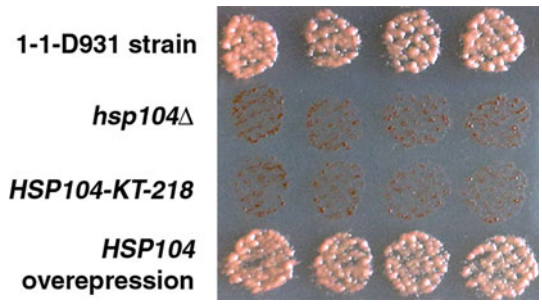

Fig. 4 Effects of Hsp104 on [NSI^+] maintenance. The 3-1-1-931 strain with the *hsp104* deletion was obtained as described in "Materials and methods". To inactivate and overproduce Hsp104, the 1-1-D931 strain was transformed with pFL39-GPD-HSP104-KT and pLH105 plasmid, respectively. After the three passages, the pFL39-GPD-HSP104-KT and pLH105 plasmids were eliminated. Hsp104-treated cells were grown on YPD + 150 μM $CuSO_4$ and replica-plated on –Ade + 150 μM $CuSO_4$ medium. Pictures of plates were taken after 3 days of incubation at 30°C

bearing *Aβ-SUP35MC* and *SUP35MC*, respectively, were transformed with the pRS424-GPD-HSP104-KT plasmid, containing a mutant copy of *HSP104*, which has a dominant-negative effect on the ATPase activity of native Hsp104 (Parsell et al. 1991). Next, the transformants were replica-plated three times on –Trp + 150 μM $CuSO_4$ medium, and the pRS424-GPD-HSP104-KT plasmid was eliminated. The temporary inactivation of Hsp104, as well as deletion of the *HSP104* gene, caused the elimination of the nonsense suppression in 90 of 100 clones of 1-1-D931 strain (Fig. 4) and in 96 of 100 clones of 2-1-1-D931 strain (not shown). Overproduction of Hsp104 causes the elimination of [PSI^+] and [*MCA*] (Chernoff et al. 1995; Nemecek et al. 2009) but not of other yeast prions (Derkatch et al. 2000; Moriyama et al. 2000; Du et al. 2008). *HSP104* overexpression in our system did not affect the nonsense suppressor phenotype (Fig. 4). Thus, [NSI^+], like all known amyloid-based yeast prions, can be cured by *HSP104* deletion and inactivation.

[NSI^+] reappears after curing and shows non-Mendelian inheritance and cytoplasmic infectivity

Prions are the only genetic elements that reappear after curing. To monitor the de novo appearance of [NSI^+], we performed a fluctuation test, as described in "Materials and methods". [NSI^+] was originally detected in the [PIN^+] strain. Taking into consideration that [PIN^+] can affect the induction frequency of [NSI^+], we analyzed the de novo appearance of [NSI^+] in [nsi^-][PIN^+] and [nsi^-][pin^-] strains. The [nsi^-][pin^-] 1-4-1-1-D931 strain was obtained by GuHCl treatment of [NSI^+][PIN^+] 4-1-1-D931 cells. The [PIN^+] factor was introduced into GuHCl-treated cells through a recently developed protein transformation assay (Tanaka and Weissman 2006; Patel and Liebman 2007). The [nsi^-][pin^-] spheroplasts were transformed with protein extract from the [PIN^+] BY4742 strain and with the pmCUPNMsGFP plasmid, bearing *SUP35NM-GFP*. The transformants were selected on –Leu–Ura + 150 μM $CuSO_4$ medium, containing 1 M sorbitol. Sup35NM-GFP fusion protein is known to form aggregates in [PIN^+] but not in [pin^-] cells (Chernoff et al. 2002). [PIN^+] cells that contained Sup35NM-GFP aggregates were identified by fluorescent microscopy. To confirm the [nsi^-] status, we verified that selected [PIN^+] cells did not grow on –Ade + 150 μM $CuSO_4$ medium. The de novo appearance of single [NSI^+] clones was detected at a rate of approximately 5×10^{-6} in the [nsi^-][PIN^+] strain but not in the [nsi^-][pin^-] strain (Table 2). These data show that [NSI^+], like other yeast prions, reappears after curing, but we cannot conclude that [PIN^+] facilitates [NSI^+] appearance because the difference in frequencies of [NSI^+] induction between [PIN^+] and [pin^-] strains is not statistically significant according to Mann–Whitney *U* test.

To determine whether the inheritance of [NSI^+] is non-Mendelian, we obtained the diploid from a cross of the isogenic 1-1-D931 MATa [NSI^+] [pU-Aβ-Sup35MC] and 2-1-1-1-D931 MATα [nsi^-] [pL-Aβ-Sup35MC] strains. The resulting diploids grew on –Ade + 150 μM $CuSO_4$ on the second day of incubation. Five independent diploid clones were sporulated and analyzed by random spore analysis. The tetrad analysis was not applicable due to low spore viabilities (none of the 70 tetrads produced 4 viable spores). The random spore analysis showed that 97–100% of spores possessed the suppressor phenotype that was

Table 2 Spontaneous de novo reappearance of [*NSI*$^+$]

The 1-4-1-1-D931 [*nsi*$^-$][*pin*$^-$] strain was obtained by GuHCl treatment of the 4-1-1-D931 [*NSI*$^+$][*PIN*$^+$] strain. The 2-4-1-1-D931 strain is the [*nsi*$^-$][*PIN*$^+$] derivative of the 1-4-1-1-D931 [*nsi*$^-$][*pin*$^-$] strain. The [*PIN*$^+$] factor was introduced into GuHCl-treated cells by protein transformation assay

Strain	No. of cells analyzed	No. of Ade$^+$ clones			
		Unstable in mitosis	Stable in mitosis		Total
			GuHCl non-curable	GuHCl curable ([*NSI*$^+$])	
2-4-1-1-D931 [*nsi*$^-$][*PIN*$^+$]	2×10^4	2	1	0	88
	2×10^5	7	12	3	
	2×10^6	25	30	8	
1-4-1-1-D931 [*nsi*$^-$][*pin*$^-$]	2×10^4	0	2	0	66
	2×10^5	4	14	0	
	2×10^6	11	35	0	

Table 3 Meiotic inheritance of [*NSI*$^+$] factor (data from random spore analysis)

Diploid cells were selected on –Leu–Ura medium and streaked for single colonies on the same medium

Diploid	No. of clones	No. of haploid segregants		Mating type ratio a:α
		[*NSI*$^+$] (Ade$^+$ Trp$^+$)	[*nsi*$^-$] (Ade$^-$ Trp$^-$)	
1-1-D931 [*NSI*$^+$] × 2-1-1-1-D931 [*nsi*$^-$]	1	29	0	14 a:15 α
	2	31	1	17 a:15 α
	3	32	1	16 a:17 α
	4	29	0	17 a:12 α
	5	33	0	19 a:14 α
2-1-1-D931 [*NSI*$^+$] × 2-2-1-1-D931 [*nsi*$^-$]	1	98	2	54 a:44 α
	2	114	1	53 a:61 α

curable by GuHCl (Table 3). In a control experiment, when two [*nsi*$^-$] strains were crossed, the diploids remained [*nsi*$^-$] and did not generate any Ade$^+$ spores after meiosis (225 ascospores were analyzed). The dominant inheritance of [*NSI*$^+$] was also shown, when we crossed 2-1-1-D931 [*NSI*$^+$] and 2-2-1-1-D931 [*nsi*$^-$] strains bearing plasmids encoding *SUP35MC* fragment. Almost all (98–99%) haploid segregants obtained from this diploid were [*NSI*$^+$] (Table 3). To circumvent the low spore viability, we have constructed the 1-D934 and 2-D934 [*NSI*$^+$] haploid strains containing the *SUP35 P.m.* gene integrated into the chromosome instead of endogenous *SUP35 S.c.* gene (see "Materials and methods"). These strains show the suppressor phenotype on –Ade medium. The GuHCl-treated derivative of the 1–D934 strain was mated to the 2-D934 [*NSI*$^+$] strain, followed by sporulation and dissection of resulting diploids. In tetrads that produced four surviving spores, the ratio of Ade$^+$:Ade$^-$ was 4:0 (Table 4). The suppressor phenotype of the spores was curable by GuHCl. In a control experiment, the 1-D934 and 2-D934 haploids were treated with GuHCl and mated to each other. The resulting diploid, D936, was sporulated and dissected. None of the Ade$^+$ spore clones was detected (Table 4). These data show that [*NSI*$^+$] demonstrates dominant non-Mendelian inheritance.

Yeast prions and some other nonchromosomal determinants are transmissible by cytoduction (abortive mating, resulting in cytoplasmic exchange) (Wright and Lederberg 1957; Zaharov et al. 1969; Conde and Fink 1976). Cytoduction is a routine test for the prion infectivity in yeast systems. Cytoplasmic determinants are transmitted by cytoduction with 100% efficiency (Cox et al. 1988), whereas cytoplasmic infectivity of nuclear prions is less efficient (Du et al. 2008; Patel et al. 2009). To determine whether [*NSI*$^+$] is infectious at the cytoplasmic level, the [*NSI*$^+$] strains 1-1-D931 and 2-1-1-D931 bearing *Aβ-SUP35MC* and *SUP35MC* genes, respectively, were mated to the karyogamy-deficient recipient [*nsi*$^-$] strains 1-D932 and 1-2-1-1-D931. The recipient strains lacked mitochondrial DNA ([*rho*^{0}]) and carried a recessive mutation that conferred cycloheximide resistance (*cyh*^{R}). Recipient cells that acquired donor cytoplasm (cytoductants) were rescued on the non-fermentable medium as they became [*rho*$^+$], i.e. acquired mitochondria. Cytoductants were colony-purified and replica-plated on a set of selective media. The cytoduction of [*NSI*$^+$] was detected at the frequency of 8–14% in strains containing deleted or modified N-terminal domain of *SUP35* (Table 5). As in the original [*NSI*$^+$] strains, the suppressor phenotype of the cytoductants was GuHCl curable. No [*NSI*$^+$] colonies were detected in the control experiments, when the [*nsi*$^-$] donor strains were mated to the [*nsi*$^-$] recipients (Table 5).

To confirm [*NSI*$^+$] infectivity, we have also used a protein transformation assay (Tanaka and Weissman 2006).

Table 4 Meiotic inheritance of [*NSI*$^+$] factor (data from tetrad spore analysis)

Diploid	No. of tetrads	No. of tetrads with Ade$^+$:Ade$^-$ segregation				
		4:0	3:1	2:2	1:3	0:4
D935 [*NSI*$^+$]	12	12	0	0	0	0
D936 [*nsi*$^-$]	14	0	0	0	0	14

The D935 [*NSI*$^+$] and D936 [*nsi*$^-$] diploid strains were obtained as described in "Results". Ade$^+$:Ade$^-$ segregation was analyzed only in tetrads containing four viable spores

Table 5 Cytoduction of [*NSI*$^+$] factor

Donor	Recipient	No. of experiment	No. of cytoductants		% of [*NSI*$^+$] clones
			Total	Ade$^+$ ([*NSI*$^+$])	
1-1-D931 [*NSI*$^+$]	1-D932 [*nsi*$^-$]	1	96	14	14.6 ± 3.6
		2	54	6	11.1 ± 4.3
1-1-1-D931 [*nsi*$^-$]	1-D932 [*nsi*$^-$]	1	108	0	0 + 0.3
2-1-1-D931 [*NSI*$^+$]	2-1-D932 [*nsi*$^-$]	1	72	6	8.3 ± 3.25
1-2-1-1-D931 [*nsi*$^-$]	2-1-D932 [*nsi*$^-$]	1	71	0	0 + 0.4

The standard error of the mean is indicated

1-D933 [*nsi*$^-$] MATα spheroplasts were transformed with protein extracts from the [*NSI*$^+$] strain 4-1-1-D931 MATa and with pRS315 empty vector to provide the initial selection of competent cells. The transformants were selected on –Leu–Ura + 150 μM $CuSO_4$ medium with sorbitol, tested for mating type MATα, and replica-plated on –Ade + 150 μM $CuSO_4$ medium. The colonies with GuHCl-curable suppression were detected at a frequency of 5–7% in three independent experiments (Table 6). The [*NSI*$^+$] infectivity was also shown when we transformed 2-2-1-1-D931 [*nsi*$^-$] MATα cells bearing Sup35MC-encoding plasmid with protein extract from the [*NSI*$^+$] 2-1-1-D931 MATa strain and with pRS316 empty vector (Table 6). In control experiments, when [*nsi*$^-$] cells were transformed with [*nsi*$^-$] extract and pRS315 empty vector, no [*NSI*$^+$] clones were observed (Table 6). These data confirm the infectious properties of [*NSI*$^+$].

[NSI$^+$] does not correspond to known yeast amyloid-based prion

We have shown that [*NSI*$^+$], like all amyloid-based prions, depends on Hsp104 activity. Seven amyloid-based prion-like proteins (Sup35, Rnq1, Ure2, Swi1, Cyc8, Mca1, and Mot3) have been identified in yeast (Wickner 1994; Derkatch et al. 1997; Du et al. 2008; Alberti et al. 2009; Nemecek et al. 2009; Patel et al. 2009). The Q/N-rich domain of a New1 protein also possesses the prion properties (Osherovich and Weissman 2001). In the following experiments, we addressed the possibility that [*NSI*$^+$] is a prion isoform of previously identified prion proteins. The prion replication is known to require the continuous expression of the prion-encoding gene. The deletion of a gene encoding prion-forming protein eliminates prion (Wickner et al. 1999). Six strains bearing the deletions of the prion-encoding genes (*RNQ1*, *URE2*, *CYC8*, *MCA1*, *MOT3* and *NEW1*) were mated to the 1-1-D931 [*NSI*$^+$] strain followed by sporulation and dissection of the resulting diploids. The *sup35Δ::HIS3*[pU-A*β*-SUP35MC] haploid segregants bearing the deletions of the genes mentioned above were selected. All of them exhibited GuHCl-curable growth on –Ade + 150 μM $CuSO_4$ medium (Fig. 5a). Thus, the maintenance of [*NSI*$^+$] does not require *RNQ1*, *URE2*, *CYC8*, *MCA1*, *MOT3*, or *NEW1* expression. As was shown above, [*NSI*$^+$] is unrelated to [*PSI*$^+$], because the maintenance of [*NSI*$^+$] does not depend on the N or M domain of Sup35 (see Figs. 2, 3). The *swi1* deletion was not considered in this experiment, because it is essential for viability.

It is known that overproduction of prion proteins considerably increases the frequency of their conversion into infectious isoform (Wickner 1994; Derkatch et al. 1997; Du et al. 2008; Alberti et al. 2009; Nemecek et al. 2009; Patel et al. 2009). We analyzed the effects of prion proteins overproduction in our system. The [*nsi*$^-$][*PIN*$^+$] 1-D933 strain was transformed with multicopy plasmids from the Yeast Genomic Tiling Collection that contained prion-encoding genes under their own promoters. The results in Fig. 5b show that the overproduction of prion proteins did not cause nonsense suppression. Taken together, these data clearly show that the novel non-Mendelian determinant [*NSI*$^+$] is unrelated to already identified amyloid-based prions.

Discussion

We have discovered a novel non-Mendelian determinant, which, like [*PSI*$^+$], causes nonsense suppression and may be detected in strains containing deleted or modified

Table 6 Analysis of [*NSI*$^+$] transmission by protein transformation assay

Protein extract from	Recipient strain	No. of experiment	No. of transformants		
			Total	Ade$^+$([*NSI*$^+$])	% of [*NSI*$^+$]
4-1-1-D931 [*NSI*$^+$]	1-D933 [*nsi*$^-$]	1	100	5	5.0 ± 2.2
		2	100	7	7.0 ± 2.6
		3	100	6	6.0 ± 2.4
1-4-1-1-D931 [*nsi*$^-$]	1-D933 [*nsi*$^-$]	1	100	0	0 + 0.3
		2	100	0	0 + 0.3
		3	100	0	0 + 0.3
2-1-1-D931 [*NSI*$^+$]	2-2-1-1-D931 [*nsi*$^-$]	1	89	4	4.5 ± 2.2
		2	73	5	6.8 ± 2.9
1-2-1-1-D931 [*nsi*$^-$]	2-2-1-1-D931 [*nsi*$^-$]	1	84	0	0 + 0.3
		2	115	0	0 + 0.3

The [*nsi*$^-$] 1-D933 and 2-2-1-1-D931 strains were transformed with protein extract and pRS315 or pRS316 vectors, respectively. The standard error of the mean is indicated

Sup35N prion-forming domain. It is very likely that [*NSI*$^+$] is an omnipotent suppressor, because we detected the suppression of two nonsense codons (*ade1-14*$_{UGA}$ and *trp1-289*$_{UAG}$) in our system. Interestingly, the suppression of *trp1-289*$_{UAG}$ by [*PSI*$^+$] has never been observed, despite evidence that [*PSI*$^+$] is an omnipotent suppressor.

In contrast to [*URE3*] and [*PSI*$^+$], which typically undergo cytoduction at 100% efficiency (Cox et al. 1988), the cytoplasmic transfer of [*NSI*$^+$] was detected in only 11–14% of recipient cells. However, the cytoduction of the prion-like factors [*OCT*$^+$] and [*SWI*$^+$], which have mainly nuclear location, is also less efficient than the transfer of cytoplasmic factors [*URE3*] and [*PSI*$^+$] (Du et al. 2008; Patel et al. 2009). Using a whole protein extract transformation protocol, we confirmed the infectivity of [*NSI*$^+$]. In this case, the transmission efficiency was comparable with that of [*PIN*$^+$] (Patel and Liebman 2007). It is important to mention that the cytoduction causes cytoplasm mixing, whereas in the transformation assay, the [*nsi*$^-$] cells receive the cytoplasmic and nuclear proteins from [*NSI*$^+$] cells. Low efficiency of cytoduction and relatively high efficiency of [*NSI*$^+$] transmission in case of the protein transformation assay suggests that [*NSI*$^+$] determinant has mainly nuclear location.

Remarkably, that non-Mendelian determinant [*NSI*$^+$], which controls nonsense suppression, was not identified before. It may be related to the specific genetic background that is required for [*NSI*$^+$] manifestation. According to our data, [*NSI*$^+$] manifestation is stronger in yeast strains containing the hybrid gene *Ab-SUP35MC* than in strains that bear the N-terminally truncated *SUP35* gene. Based on these data, we cannot exclude the possibility that defects in the regulation of translational accuracy enhance [*NSI*$^+$] manifestation. The expression of full-length *SUP35 S.c.*, but not *SUP35 P.m.*, masks [*NSI*$^+$] manifestation, suggesting that [*NSI*$^+$] interacts with the Sup35N-terminal domain.

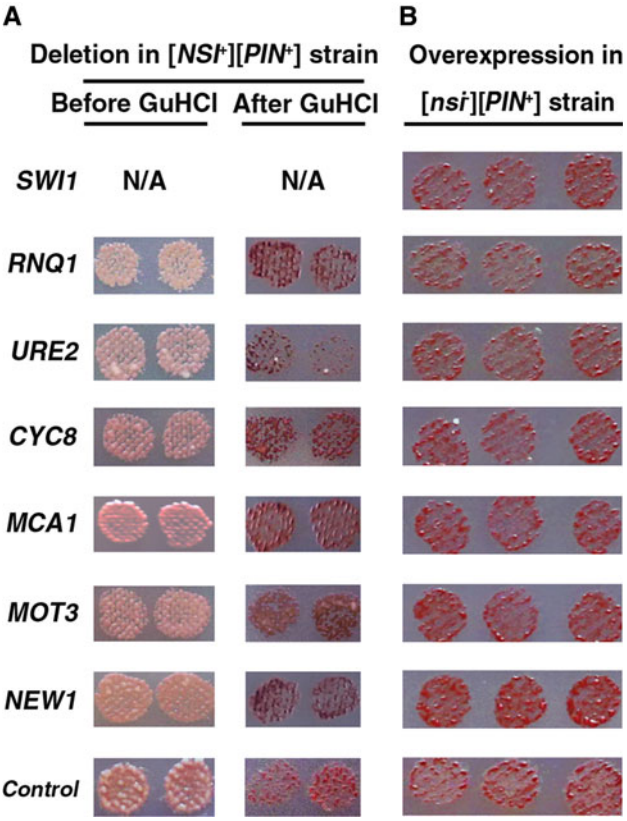

Fig. 5 Effects of prion-encoding gene deletion and overexpression on [*NSI*$^+$] maintenance and induction, respectively. **a** Growth of [*NSI*$^+$] cells containing deletions of *RNQ1*, *URE2*, *CYC8*, *MCA1*, *MOT3*, or *NEW1* on –Ade + 150 μM $CuSO_4$ medium is shown. GuHCl treatment eliminated the nonsense suppression. **b** 1-1-1-D931 [*nsi*$^-$] cells were transformed with multicopy plasmids from the Yeast Genomic Tiling Collection containing *RNQ1*, *URE2*, *SWI1*, *CYC8*, *MCA1*, *MOT3*, and *NEW1* genes. The overexpression of prion-encoding genes did not rescue cell growth on –Ade + 150 μM $CuSO_4$ medium

In 2002, the nonchromosomal determinant [*ISP*$^+$], which is an antisuppressor of certain *sup35* mutations, was described (Volkov et al. 2002). Thus, [*NSI*$^+$] is the third non-Mendelian determinant, apart from [*PSI*$^+$] and [*ISP*$^+$],

that affects the efficiency of nonsense suppression in yeast. The identification and further characterization of [*NSI*$^+$] factor may be important in increasing of our understanding of nonsense suppression epigenetic control.

We have shown that [*NSI*$^+$] possesses certain features of yeast prions, such as reversible curability, non-Mendelian inheritance, and cytoplasmic infectivity. [*NSI*$^+$], similar to amyloid-based yeast prions, but unlike DNA- and RNA-based non-Mendelian determinants, can be cured by *HSP104* inactivation or deletion, and reappears after curing. Considering that overexpression and deletion of the genes that encode known yeast prions does not affect [*NSI*$^+$] induction or maintenance, we propose that [*NSI*$^+$] is a novel prion factor. A yeast proteomic screen to identify [*NSI*$^+$] determinants is in progress.

To conclude, we have shown that novel non-Mendelian determinant described here possesses certain characteristics of yeast prion and is involved in the epigenetic control of nonsense suppression.

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