et al., *Transgenic Res.* **1**, 285 (1992)] carried within the *DsG* element. In addition, the *DsG* element contained a *gusA* reporter gene in opposite orientation to the *nptII* gene (Fig. 2A). Note that the *nptII* gene is contained within the *Ds* element, not within the flanking T-DNA. Seedlings carrying independent transposed elements were transplanted to soil and allowed to self-fertilize, giving rise to a gene trap line. The seeds from each line were sown on agar and stained for GUS activity 3 to 7 days after germination.
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18. Complementary DNA synthesis was carried out with 10 μg of total RNA isolated from flowers of *prl* heterozygous plants and GUS4 primer (5′-GCTCTAGATCGGCGAACTGATCGTTAAAAC-3′). Second-strand synthesis and polymerase chain reaction (PCR) were carried out with oligo(dT)$_{17}$-adaptor and Adaptor primers (*17*) and GUS3 primer (5′-GAGAATTCTTGTAACGCGCTTTCCCACCA-3′). PCR reactions were heated to 95°C for 5 min, followed by 40 cycles of amplification (95°C, 1 min; 53°C, 1 min; 72°C, 1.5 min) and 10-min final extension at 72°C. Products were size-fractionated, reamplified with Adaptor and GUS3 primers, gel-purified, and cloned into M13.
19. Complementary DNA synthesis was carried out with 5 μg of total RNA isolated from floral buds of wild-type plants and primer 148C (5′-GGGAGCTCTTGCATCTTGGCCTCCTGAAAC-3′). Second-strand cDNA synthesis and PCR were done with the Q_T and Q_O primers [M. A. Frohman, *Methods Enzymol.* **218**, 340 (1993)] and primer 148B (5′-GGGAATTCCCGGCTTTACTATTAAGACG-3′). Reamplification was done with Q_I and 148B primers. Amplified products were gel-purified after size selection and cloned into pBluescript.
20. Double-stranded template DNA was isolated with the use of Qiawell strips (Qiagen, Inc., Chatsworth, CA) and previously described modifications of the Qiagen protocol [W. R. McCombie, C. Heiner, J. M. Kelley, M. G. Fitzgerald, J. D. Gocayne, *DNA Sequencing* **2**, 289 (1992)]. Cycle sequencing reactions were carried out with fluorescent forward and reverse primers (Applied Biosystems, Foster City, CA) and SequiTherm polymerase (Epicenter Technology, Madison, WI). The sequences of overlapping clones were assembled with the use of Xbap software [T. J. Gleeson and R. Staden, *Comput. Appl. Biosci.* **7**, 398 (1991)]. Gaps remaining after assembly of the subcloned sequences were filled with custom primers and either Sequenase dye terminator or Taq dye terminator kits (Applied Biosystems). All sequence data were acquired through use of ABI373 DNA sequencers with 34-cm stretch liners (Applied Biosystems). Databases were searched with the Blast program [S. F. Altschul, G. Warren, W. Gish, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)].
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27. Primers 148AR and 148D, which flank the *DsG* insertion in the cDNA sequence, were used to amplify intron sequences from genomic DNA from wild-type and revertant plants. The DNA sources used for the amplification are the same as those used in Fig. 4A, lanes 1 (wild type) and 3 (revertant) (*29*). Primers Ds3O2 and 148AR, and Ds5O and 148D were used to amplify sequences flanking the *DsG* insertion from genomic DNA from *prl* heterozygotes. The genomic DNA source was the same as that used in Fig. 4A, lane 2 (*29*). 148AR, 5′-AACTGCAGATGCCCTGAACTGGATCTCCA-3′; 148D, 5′-AGCTGCAGATTCCTCCAAACACTC-3′; Ds3O2, 5′-CGACCGGTACCGACCGTTTTCATCC-3′; and Ds5O, 5′-GTTCGAATTCGATCGGGGATAAAAC-3′. Amplified products were gel-purified, cloned into M13, and sequenced.
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29. Fully fertile and semi-sterile siliques were harvested from plant 724-12 (*prl*/+, *Ac*/+). Seed from semi-sterile siliques was selected on kanamycin (selects for the presence of the *Ds* element) and NAM (selects against the *iaaH* gene which is linked to *Ac*) (*13*). Eight survivors were transferred to soil, and leaf tissue from each plant was collected and pooled to isolate DNA shown in lanes 2 and 6. Seed from single fully fertile siliques was planted, and tissue from individual plants was collected and pooled, to isolate DNA shown in lanes 3 and 7 (55 plants) and lanes 4 and 8 (40 plants). DNA was isolated as described [K. Cone, *Maize Genetics Coop. Newsletter* **63**, 68 (1989)].
30. GUS activity was localized by staining at 37°C in 1 mM 5-bromo-4-chloro-3-indolyl β-D glucuronic acid (X-Gluc, Biosynth AG) for 1 to 2 days by a modification of standard methods [R. A. Jefferson, T. A. Kavanagh, M. W. Bevan, *EMBO J.* **6**, 3901 (1987)]. Tissue was vacuum-infiltrated for 15 min in a solution containing 0.1 M $NaPO_4$ buffer (pH 7.0), 10 mM EDTA (pH 7.0), 0.1% Triton X-100, 1 mM X-Gluc, and chloramphenicol (100 μg/ml). After staining, the tissue was cleared of chlorophyll by soaking in 70% ethanol.
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33. The sequences were aligned with the Pileup and Prettybox programs [Genetics Computer Group, *Program Manual for the GCG Package*, *Version 7* (Madison, WI, 1991)] with a gap weight of 5 and a length weight of 0.1.
34. The gene trap tagging system was developed by V.S. and R.A.M. in collaboration with H. Ma, J. Jones, and C. Dean. We thank J. Colasanti, U. Grossniklaus, H. Ma, and B. Stillman for helpful comments on the manuscript; H. Cross, L. Das, J. Montagu, T. Mulligan, and T. Volpe for technical assistance; D. Weigel for providing the flower cDNA library; and U. Grossniklaus for suggesting the name *PROLIFERA*. Supported by the Robertson Research Fund; National Science Foundation grant MCB-9408042 to R.A.M., V.S., and W.R.M; and National Science Foundation Postdoctoral Research Fellowship in Plant Biology BIR-9303612 to P.S.S.

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Role of the Chaperone Protein Hsp104 in Propagation of the Yeast Prion-Like Factor [*psi*$^+$]

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The yeast non-Mendelian factor [*psi*$^+$] has been suggested to be a self-modified protein analogous to mammalian prions. Here it is reported that an intermediate amount of the chaperone protein Hsp104 was required for the propagation of the [*psi*$^+$] factor. Overproduction or inactivation of Hsp104 caused the loss of [*psi*$^+$]. These results suggest that chaperone proteins play a role in prion-like phenomena, and that a certain level of chaperone expression can cure cells of prions without affecting viability. This may lead to antiprion treatments that involve the alteration of chaperone amounts or activity.

Certain mammalian neurodegenerative diseases, such as sheep scrapie, human Creutzfeldt-Jacob disease, and bovine spongiform encephalopathy, are widely believed to be caused by proteins in an unusual protease-resistant conformation, called prions (*1*). Evidence indicates that transmission of the prion diseases does not require any nucleic acid. Rather, infection depends on the prion's ability to convert unmodified nonprion protein, encoded by the same host gene, into the prion conformation. It has been noted that the yeast factors [*URE3*] (*2*) and [*psi*$^+$] (*2*, *3*) closely resemble prions.

The [*psi*$^+$] factor was first described as a non-Mendelian element, found in some but not other laboratory strains of *Saccharomyces cerevisiae*, that increases the efficiency of certain nonsense suppressor transfer RNAs [for a review, see (*4*)]. Later it was shown that [*psi*$^+$] causes weak nonsense suppression by itself (*4*–*6*). No extrachromosomal DNA or RNA elements have been found to be responsible for [*psi*$^+$] (*4*). Moreover, [*psi*$^+$] can be "cured" (lost from the cell) by stress-inducing agents (*4*). Several pieces of evidence strongly suggest (*2*, *3*) that [*psi*$^+$] is a prion-like form of the EF-1α–related protein Sup35. (i) Mutations in the *SUP35* gene cause omnipotent suppression, just as [*psi*$^+$] does (*7*). (ii) Overexpression of *SUP35* induces [*psi*$^+$] (*8*), just as overexpression of the prion protein gene (*PrP*) in transgenic mice induces the prion disease

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(9). (iii) The 5′ coding region of the *SUP35* gene is required for the maintenance of [*psi*$^+$] (*10*), just as the *PrP* gene is required for the maintenance of the mouse prion (*11*). (iv) The corresponding NH_2-terminal domain of the Sup35 protein shows a remarkable structural similarity to mammalian prions, including a region of tandem oligopeptide repeats (*3*), mutations in which cure [*psi*$^+$] (*3*, *10*).

Here, we searched for extra copy modifiers of [*psi*$^+$]-mediated nonsense suppression. The [*psi*$^+$] strain YC13-6C (Table 1) contained *sup111*, which increases the suppression efficiency of [*psi*$^+$] (*6*). In the presence of [*psi*$^+$], nonsense mutations *leu2-1* (UAA), *ilv1-2* (UAA), and *met8-1* (UAG) were suppressed, leading to cell growth on media lacking leucine, isoleucine and valine, or methionine, respectively (see Table 1). Among more than 6000 centromeric (*CEN*) plasmids screened from a YCp50-based *S. cerevisiae* genomic library, one inhibited [*psi*$^+$]-mediated suppression in strain YC13-6C (*12*). Deletion analysis of this clone and partial sequencing of the minimal functional subclone (*12*) revealed that it contained a gene coding for the heat shock protein Hsp104. Furthermore, we found that a *CEN* plasmid pYS104 containing the independently cloned and sequenced *HSP104* gene (*13*) also had an inhibitory effect on [*psi*$^+$]-mediated suppression. The inhibition of suppression caused by the *HSP104*-containing plasmid was neither strain-specific nor *sup111*-specific because it was reproduced in other [*psi*$^+$] strains that did not contain *sup111* (see Table 1 and Fig. 1). In the presence of pYS104, [*psi*$^+$] was not able to suppress nonsense mutations (Fig. 1). However, suppression was restored in cells that lost the plasmid, suggesting that [*psi*$^+$] is inhibited but not eliminated by pYS104. Protein immunoblot analysis (Fig. 2) confirmed that the Hsp104 protein was overaccumulated two- to fourfold in strains bearing the pYS104 plasmid compared with strains lacking the plasmid. The same plasmid containing a mutant *hsp104* allele, truncated by introducing two in-frame nonsense codons into the NH_2-terminal region, did not cause overaccumulation of the Hsp104 protein in [*psi*$^+$] strains (*14*) and did not inhibit [*psi*$^+$]-mediated suppression (Fig. 1). Thus, it is clear that it was excess Hsp104 protein and not *HSP104* DNA or another component of the plasmid that was required for the inhibition of [*psi*$^+$].

Several mutant derivatives of *HSP104* were also tested for their interactions with [*psi*$^+$]. Yeast Hsp104 protein is an adenosine triphosphotase (ATPase) and contains two ATP-binding sites (*15*). Mutations in either site ($Lys^{218}\rightarrow Thr^{218}$ or $Lys^{620}\rightarrow Thr^{620}$) compromise Hsp104 function in thermotolerance, particularly in the disaggregation of heat-damaged proteins (*15*). Apparently, the use of ATP is essential for the chaperone function of Hsp104. A *CEN* plasmid, bearing a mutant *hsp104-KT218,620* allele (K and T refer to Lys and Thr, respectively) with both ATP-binding sites inactivated, converted [*psi*$^+$] strains into [*psi*$^-$] strains. In this case, [*psi*$^+$]-mediated suppression did not reappear after the loss of the plasmid, suggesting that mutant Hsp104 cured rather than just inhibited [*psi*$^+$] (Fig. 1). Because the double mutation, *hsp104-KT218,620*, caused the loss of [*psi*$^+$] in the presence of the chromosomal wild-type *HSP104* gene, it is a dominant *psi no more* (*PNM*) (*3*, *4*) mutation. Alleles of *hsp104*, in which only one of the two ATP-binding sites was inactivated, each showed strain-dependent effects: *hsp104-KT218* cured [*psi*$^+$] in strains 74-D694 and YC13-6C and inhibited but did not cure [*psi*$^+$] in strain D1142-1A, whereas *hsp104-KT620* cured [*psi*$^+$] only in strain 74-D694 and inhibited [*psi*$^+$] in two other strains (*16*).

The dominant effect of *hsp104-KT218,620* may have resulted from a competition between the mutant (nonfunctional) and wild-type (functional) Hsp104 protein. The multimeric organization of Hsp104 (*15*) may facilitate such a dominant negative effect. To determine whether the absence of functional Hsp104 affects the propagation of [*psi*$^+$], we disrupted *HSP104* in the three different [*psi*$^+$] strains listed in Table 1. Protein immunoblot analysis (Fig. 2) confirmed the absence of Hsp104 protein in the disruptant strains. All *hsp104-Δ* (*hsp104::URA3* and *hsp104::LEU2*) disruptants lost [*psi*$^+$] (Fig. 1). Two indepen-

Table 1. Genotypes of yeast strains. The strains YC13-6C (*6*, *12*) and D1142-1A (*27*) were originally [*psi*$^+$]. [*Psi*$^-$] derivatives of these strains were obtained by growth in the presence of 5 mM guanidine hydrochloride, as described (*4*). The strain 74-D694 was originally [*psi*$^-$]. A [*psi*$^+$] derivative of this strain was obtained by growth in the presence of overexpressed *SUP35*, as described (*8*). As previously noted (*4*), [*psi*$^+$]-mediated suppression varied in efficiency and was usually detected after 2 to 8 days of incubation on standard selective media (*28*), depending on both the strain and marker. No suppression was detected in [*psi*$^-$] strains.

Strain	Genotype	Markers suppressed in [*psi*$^+$]
YC13-6C	*MATα ade2 aro7 ilv1 leu2 met8 trp1 ura3 can1 sup111* [*psi*$^+$] or [*psi*$^-$]	*met8-1* (UAG) *ilv1-2* (UAA)* *leu2-1* (UAA)*
D1142-1A	*MATa aro7 cyc1 his4 leu2 lys2 met8 trp5 ura3* [*psi*$^+$] or [*psi*$^-$]	*met8-1* (UAG) *trp5-48* (UAA) *lys2-187* (UGA)
74-D694	*MATa ade1 his3 leu2 trp1 ura3* [*psi*$^+$] or [*psi*$^-$]	*ade1-14* (UGA)

*These markers are not suppressed in [*psi*$^+$] strains lacking *sup111*.

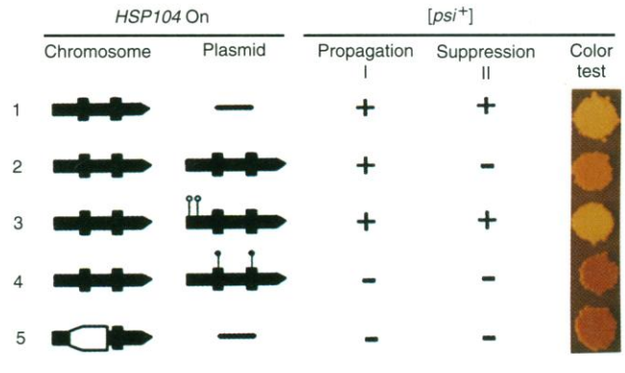

Fig. 1. Propagation and expression of [*psi*$^+$] in strains that differ in *HSP104* gene copy number. ▬, *HSP104* gene with two ATP binding sites (ABSs). Constructs 1 through 4 each contain one wild-type chromosomal copy of *HSP104* and bear the following [*URA3 CEN*] plasmids: construct 1, pRS316 (*29*); construct 2, pYS104 (*13*) which is pRS316 plus *HSP104*; construct 3, pTerm-104 (*14*) which is pYS104 with two nonsense mutations (♀) introduced shortly after the ATG codon of *HSP104*; and construct 4, pKT218,620 (*15*) which is pYS104 with two missense mutations (♂), $Lys^{218}\rightarrow Thr^{218}$ and $Lys^{620}\rightarrow Thr^{620}$, that inactivate the NH_2-terminal and the COOH-terminal ABS, respectively. Construct 5 contains a disruption of the chromosomal *HSP104* gene, *hsp104-Δ* (*30*), and does not carry a plasmid. All constructs were tested in all the [*psi*$^+$] strains listed in Table 1, and the results were the same for all strains and all suppressible markers. The experimental procedures used (*31*) allowed us to distinguish between the inability of the cells to propagate [*psi*$^+$] (constructs 4 and 5) and the inhibition of [*psi*$^+$]-mediated suppression (construct 2). I and II refer to the experimental assay used (*31*). Strain 74-D694 also provides a color assay for [*psi*$^+$]. After 2 to 3 days of incubation on complete medium (YPD), [*psi*$^-$] derivatives were red, whereas [*psi*$^+$] derivatives were white because of partial suppression of the gene *ade1-14*. The color test results were in good agreement with growth test results: Both pRS316 (construct 1) and pTerm-104 (construct 3) transformants were white ([*psi*$^+$]), the pYS104 transformant (construct 2) was pink because it is a mixture of red sectors containing the plasmid that inhibits [*psi*$^+$] and white ([*psi*$^+$]) sectors that lost the plasmid, and both the pKT218,620 transformant (construct 4) and the *hsp104-Δ* disruptant strain (construct 5) were red ([*psi*$^-$]).

dent *hsp104::URA3* [*psi*$^-$] derivatives of YC13-6C were crossed to the [*psi*$^+$] strain D1142-1A. The resulting diploids were [*psi*$^+$], judging from the suppression of the homozygous *met8-1* mutation, which is efficiently suppressed by [*psi*$^+$] (6). This means that a heterozygous disruption of *hsp104* has no effect on [*psi*$^+$]. The diploids were sporulated, and among 10 tetrads analyzed, all *hsp104::URA3* (Ura$^+$) spores were [*psi*$^-$], whereas all *HSP104*$^+$ (Ura$^-$) spores were [*psi*$^+$]. In the control isogenic [*psi*$^+$] diploid that was homozygous for *HSP104*$^+$, [*psi*$^+$] segregated 4+:0−, as usual (4). To verify the absence of [*psi*$^+$] in the *hsp104::URA3* disruption segregants, we crossed them to [*psi*$^-$] *HSP104*$^+$ *met8-1* strains. None of the resulting *HSP104*$^+$/*hsp104::URA3* heterozygous diploids were [*psi*$^+$], indicating that [*psi*$^+$] was actually lost and not just inhibited in the *hsp104::URA3* spores. Thus, an *hsp104-Δ* disruption is a recessive *PNM* mutation.

Conversion of yeast strains from [*psi*$^-$] to [*psi*$^+$] can normally be achieved by the overproduction of Sup35 protein (8). To determine whether it is possible to induce [*psi*$^+$] de novo in the absence of Hsp104, we transformed both *HSP104*$^+$ [*psi*$^-$] and *hsp104-Δ* [*psi*$^-$] versions of strain 74-D694 with a multicopy plasmid bearing the wild-type *SUP35* gene. Multicopy *SUP35* caused nonsense suppression and induced [*psi*$^+$] in the *HSP104*$^+$ strain. However, both suppression and [*psi*$^+$] induction were absent in the isogenic *hsp104*-disrupted strain (Fig. 3).

Thus, the lack of functional Hsp104 protein caused the loss of [*psi*$^+$], whereas an approximately two- to fourfold increase in the amount of Hsp104 protein inhibited suppression caused by [*psi*$^+$]. Growth temperature affects [*psi*$^+$]-mediated suppression (8, 17), but it is unlikely that Hsp104 functions directly in translational termination, because suppression caused by either the ribosomal protein gene mutation *SUP44* or the aminoglycoside antibiotic paromomycin (7) is unaffected by Hsp104 amounts in [*psi*$^-$] strains (16). Rather, we propose that an intermediate amount of the functional Hsp104 protein is required for the propagation of [*psi*$^+$]. If so, we might expect even transient expression of a large excess of Hsp104 protein to cure [*psi*$^+$]. To check this, we transformed the [*psi*$^+$] strains D1142-1A and 74-D694 with a *CEN* plasmid bearing *HSP104* under the control of the galactose-inducible (*GAL*) promoter. On glucose medium, little or no Hsp104 protein was expressed from this plasmid, whereas on galactose Hsp104 was expressed at about the same level as it is normally expressed in a wild-type strain (with no plasmid) after heat shock (18). If the cells were never grown on galactose, the *GAL::HSP104* plasmid did not affect [*psi*$^+$]. However, after incubation on galactose, [*psi*$^+$] strains bearing this plasmid became [*psi*$^-$] (Fig. 4). The loss of [*psi*$^+$] was confirmed from the observation that neither a shift from galactose to glucose nor the loss of the plasmid restored suppression. We conclude that overexpression of *HSP104* cured the yeast cells of [*psi*$^+$].

These results may explain the findings that both environmental stresses, such as exposure to heat or ethanol, and some conventional mutagens, such as ultraviolet (UV) light and methyl methanesulphonate (MMS), can cure [*psi*$^+$] (4). This is because induction of Hsp104 is a component of the stress response. In particular, Hsp104 is shown to be induced by heat, ethanol (13),

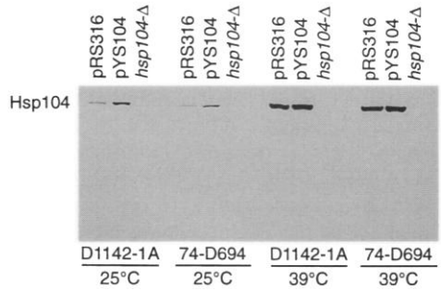

Fig. 2. Hsp104 protein amounts in strains that differ in *HSP104* gene copy number. Yeast cultures were grown in –Ura medium to the middle of the logarithmic phase at 25°C, and for 45 min the cultures were either maintained at that temperature or shifted to 39°C to induce Hsp expression. Proteins were extracted, separated in 7% SDS-polyacrylamide gels, transferred to Immobilon filters (Millipore), and reacted with an antibody specific for Hsp104 (#8-1) as described (15). Coomassie staining (32) before immunological reaction confirmed that the amount of total protein did not vary significantly among the samples. Immunochemical data shown on the figure confirmed that [*URA3 HSP104*] (pYS104) transformants contain from two- to fourfold more Hsp104 protein than the control [*URA3*] (pRS316) transformants under normal growth conditions, whereas *hsp104::URA3* disruptant strains (*hsp104-Δ*) do not contain any Hsp104 protein even after heat induction. Stationary phase cells bearing pYS104 (32) also contained three- to fourfold more Hsp104 protein than those bearing pRS316. The experiment was reproduced three times with similar results.

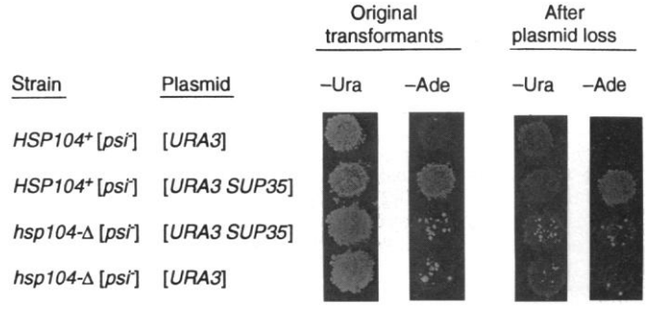

Fig. 3. Effects of multiple copies of the *SUP35* gene in *HSP104*$^+$ and *hsp104-Δ* strains. *HSP104*$^+$ [*psi*$^-$] refers to the [*psi*$^-$] 74-D694 strain (Table 1). A derivative of [*psi*$^+$] 74-D694, in which [*psi*$^+$] was lost as result of *hsp104::LEU2* disruption (30), is listed as *hsp104-Δ* [*psi*$^-$]. Both the disrupted and nondisrupted strains were transformed with either the episomal [*URA3*] plasmid pEMBLyex4 or its derivative containing a wild-type *SUP35* gene (33). The original transformants were replica plated onto medium lacking uracil (–Ura) or adenine (–Ade). A suppressor effect caused by multiple copies of *SUP35* was detected by growth on –Ade medium as a result of suppression of the *ade1-14* (UGA) mutation. [*Psi*$^+$] induction by multicopy *SUP35* was detected as residual suppression of *ade1-14* in transformants after plasmid loss, in which the [*URA3*]-containing plasmid was cured by counterselection on 5-fluoroorotic acid (5-FOA) (28). The difference in papillation between *hsp104-Δ* and *HSP104*$^+$ strains, shown here, was not reproducible. To distinguish between the absence of [*psi*$^+$] and the inability of [*psi*$^+$] to be expressed in an Hsp104$^-$ background, we crossed plasmid-cured transformants of an *hsp104-Δ* strain to a [*psi*$^-$] *HSP104*$^+$ *ade1-14* strain. The absence of suppression of *ade1-14* in the resulting diploids (32) indicates that the [*psi*$^+$] factor was not present in either parent.

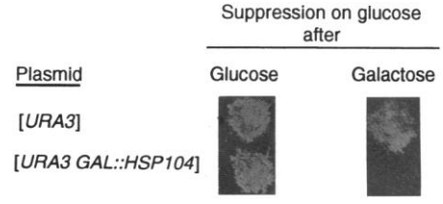

Fig. 4. Elimination of the [*psi*$^+$] factor caused by overexpression of the *HSP104* gene. Yeast strains were transformed with either pRS316 ([*URA3*]) or pYS-GAL104 ([*URA3 GAL::HSP104*]) plasmids. Plasmid pYS-GAL104 (18) is identical to pYS104 except that it contains a *GAL* promoter instead of the normal *HSP104* promoter. Transformants were grown on glucose medium lacking uracil and were then replica plated to either the same medium (glucose) or the same medium containing galactose instead of glucose (galactose). After 3 days of incubation, transformants from each medium were replica plated to glucose medium deficient in both uracil and the nutrient corresponding to the marker to be tested for suppression. A color test on YPD medium (see legend to Fig. 1) was also used for 74-D694 (32). In the presence of pYS-GAL104 both D1142-1A and 74-D694 lost [*psi*$^+$] after incubation on galactose but not on glucose. Suppression of *trp5-48* in D1142-1A is shown as an example.

and MMS (*19*), and Hsp104-dependent thermotolerance is induced by radiation (*20*). Furthermore, the observation that DNA damage, caused by UV and other related mutagens, induces expression of at least some stress-related proteins (*21*) may explain why DNA repair can reverse the effect of UV light on [*psi*$^+$] loss (*4*).

Growth in the presence of guanidine hydrochloride (GuHCl) is the most efficient treatment reported for curing [*psi*$^+$] (*4*). This effect has been attributed to the protein denaturing activity of GuHCl on Sup35 (*2*, *3*). However, the 5 mM concentration of GuHCl commonly used to cure [*psi*$^+$] is too low to cause significant denaturation of most cellular proteins. We suggest that GuHCl acts on [*psi*$^+$] indirectly, by changing the amount of heat shock proteins. Indeed, the amount of Hsp104 protein was selectively increased about three- to fivefold by growth in the presence of 5 to 10 mM GuHCl (*22*). Although it is generally believed that yeast strains cured of [*psi*$^+$] by 5 mM GuHCl are very stable (*4*), in some of them [*psi*$^+$] frequently reappears de novo (*8*, *17*). One possible explanation is that these differences in the frequency of [*psi*$^-$] to [*psi*$^+$] conversion are related to strain variations in basal Hsp104 expression (*18*). Another yeast non-Mendelian prion-like element, [*URE3*], theorized to be encoded by *URE2*, has also been reported to be cured by 5 mM GuHCl (*2*). Aigle observed (*23*), and we confirmed (*16*), that the frequency of [*URE3*] appearance is increased up to 100-fold when yeast cells were stored at low temperatures. This suggests that stress-induced functions also influence the appearance and propagation of [*URE3*].

To determine whether chaperone proteins other than Hsp104 are involved in the propagation and expression of [*psi*$^+$], we transformed yeast [*psi*$^+$] strains 74-D694 and D1142-1A with a plasmid containing *SSA1*, which encodes one of the major proteins of the Hsp70 family (*24*). In both strains [*psi*$^+$]-mediated suppression was inhibited in the presence of the *SSA1*-containing plasmid. However, suppression was restored after plasmid loss, indicating that [*psi*$^+$] was not eliminated. Thus, in parallel with the Hsp104 results, overdosage of the Hsp70-encoding gene inhibited [*psi*$^+$]. This is in good agreement with the previously reported observation that excess Hsp70 partially compensates for the loss of Hsp104 in thermotolerance assays (*25*), providing further support for a close functional relation between these proteins.

Hsp104 is a member of the Hsp100 (Clp) family which is conserved in both prokaryotes and eukaryotes (*15*). Yeast Hsp104 has a chaperone-like function and increases thermotolerance by promoting the disaggregation of proteins that have been damaged and aggregated by heat (*15*). This provides a hypothesis to explain the effect of Hsp104 on the propagation of [*psi*$^+$]. It has been proposed (*26*) that conformational conversion from the wild-type to the prion form may require a partially unfolded intermediate. A prion "template" is thought to complex with this intermediate, converting it into the prion conformation. We suggest that the Hsp104 chaperone may be required for the formation or maintenance (or both) of the partially unfolded form of the Sup35 protein. Therefore, an insufficient amount of chaperone would prevent formation of the prion. If the amount of chaperone was too high, however, the prion "template" would disaggregate from the unfolded intermediate, leading to the loss of the protein-modifying activity. The finding that chaperones are essential for the propagation of [*psi*$^+$] explains why prion formation is inefficient in vitro in the absence of chaperones and provides support for the model that protein folding is responsible for [*psi*$^+$], as well as for other prion phenomena.

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30. To construct *hsp104*-Δ disruptions, we deleted and replaced the 1.2-kb piece of the *HSP104* gene between the Apa I and Bgl II sites by either a *URA3* gene (*hsp104::URA3* disruptions) or a *LEU2* gene (*hsp104::LEU2* disruptions) in the opposite orientation. Replacements were generated as described [R. Rothstein, in *DNA Cloning: A Practical Approach*, D. M. Glover, Ed. (IRL, Oxford, 1985), vol. 2, p. 45] and verified by Southern (DNA) analysis.
31. To distinguish between the cells' inability to propagate [*psi*$^+$] and inhibition of [*psi*$^+$]-mediated suppression, we first grew yeast transformants bearing autonomous [*URA3*] plasmids (Fig. 1, constructs 1 through 4) under nonselective conditions so that a substantial fraction of the cells (typically more than 10%) lost the [*URA3*]-containing plasmid. Transformants were then replica plated onto two types of selective media [see (*28*) for recipes]. The first medium (used in assay I) did not select for retention of the plasmid because it only lacked the nutrient corresponding to the marker to be tested for suppression. The other medium (used in assay II) was deficient in both this nutrient and uracil and thus selected for retention of the [*URA3*] plasmid. Transformants that cannot propagate [*psi*$^+$] (construct 4) showed no growth on either (I) or (II) media. Transformants, in which [*psi*$^+$] was unable to suppress but was not lost (construct 2), did not grow on medium (II) but grew on medium (I) because [*psi*$^+$] became active in cells that had lost the plasmid. In another assay, haploid strains bearing stable chromosomal *hsp104*-Δ disruptions (Fig. 1, construct 5) were each crossed to appropriate [*psi*$^-$] *HSP104*$^+$ strains that contain the same [*psi*$^+$]-suppressible marker or markers. If [*psi*$^+$] were unable to suppress but were still present in the *hsp104*-Δ strains, it should become active in *HSP104*$^+$/*hsp104*-Δ diploids. However, neither the resulting diploids (shown in I) nor the original haploid disruptants (shown in II) grew on media lacking nutrients corresponding to the homozygous [*psi*$^+$]-suppressible markers, indicating that [*psi*$^+$] was lost in the disruptants.
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34. We credit K. A. Kounakov with the initial observation that our [*psi*$^+$]-inhibiting DNA clone contains the *HSP104* gene. We are grateful to M. Patino for guanidine hydrochloride induction data and to E. Schirmer for the construction of pTerm-104 plasmid solely for use in this report. We thank J. Taulien and V. N. Kulikov for technical assistance; K. A. Kouna-

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KAI1, a Metastasis Suppressor Gene for Prostate Cancer on Human Chromosome 11p11.2

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A gene from human chromosome 11p11.2 was isolated and was shown to suppress metastasis when introduced into rat AT6.1 prostate cancer cells. Expression of this gene, designated *KAI1*, was reduced in human cell lines derived from metastatic prostate tumors. *KAI1* specifies a protein of 267 amino acids, with four hydrophobic and presumably transmembrane domains and one large extracellular hydrophilic domain with three potential N-glycosylation sites. *KAI1* is evolutionarily conserved, is expressed in many human tissues, and encodes a member of a structurally distinct family of leukocyte surface glycoproteins. Decreased expression of this gene may be involved in the malignant progression of prostate and other cancers.

Metastasis, the main cause of death for most cancer patients, remains one of the most important but least understood aspects of cancer (*1*). Both positive and negative regulators of metastasis are likely to exist. The existence of genes involved in metastasis suppression is suggested by somatic cell genetic studies in which nonmetastatic and metastatic tumor cells are hybridized and the resultant cell hybrids are tumorigenic but no longer metastatic (*2*). For example, the metastatic ability of rat AT6.1 prostate cancer cells was suppressed when they were fused to nonmetastatic cancer cells (*3*), and the putative metastasis suppressor gene was mapped to human chromosome 11p11.2-13 (the p11.2-13 region of chromosome 11) by microcell-mediated chromosome transfer (*4*).

To clone this metastasis suppressor gene on human chromosome 11, we isolated genomic DNA fragments from the p11.2-13 region by human-specific Alu element–mediated polymerase chain reaction (Alu-PCR) (*5*) with DNAs from the metastasis-suppressed microcell hybrid AT6.1-11-1 and from the nonsuppressed hybrids AT6.1-11-2 and AT6.1-11-3 (*4*, *6*). The Alu-PCR fragments in the AT6.1-11-1 DNA were then used as probes to screen a complementary DNA (cDNA) library from the suppressed cell hybrid clone AT6.1-11-1 that contained human chromosomal region 11cen-p13. Of five cDNA clones obtained (*7*), all were expressed in the suppressed hybrid but not in the nonsuppressed hybrids, as detected by reverse transcription PCR (RT-PCR). Northern (RNA) blot analysis (*8*) of human prostate tissue and the metastasis-suppressed hybrid cells (AT6.1-11-1) revealed that two of the cDNA clones detected 2.4-kb and 4.0-kb sequences in human tissue and in the suppressed hybrid cells, respectively. These two sequences were not detected in the parental AT6.1 cells or in the nonsuppressed hybrid cells (AT6.1-11-2 and AT6.1-11-3). The clone that detected the 2.4-kb sequence, designated *KAI1* for *kang ai* (Chinese for anticancer), was abundant in AT6.1-11-1 (Fig. 1A) and was analyzed further.

Southern (DNA) blot analysis confirmed that *KAI1* was isolated from human chromosome 11p11.2-13. Only the cell hybrids containing the p11.2-13 region involved in metastasis suppression exhibited the pattern observed with normal human DNA when hybridized to the *KAI1* probe. Fluorescence in situ hybridization of a *KAI1* probe to metaphase chromosomes further localized *KAI1* to the p11.2 region (*9*).

The *KAI1* cDNA has a single open reading frame (nucleotide positions 166 to 966) that predicts a protein of 267 amino acids with a molecular mass of 29,610 daltons (Fig. 1B). An Alu element is present in the 3′-untranslated region of the cDNA. The predicted protein has four hydrophobic and presumably transmembrane domains and one large extracellular hydrophilic domain with three potential N-glycosylation sites. A search of the GenBank and European Molecular Biology Laboratory (EMBL) databases revealed that *KAI1* is identical to three cDNA clones from human lymphocytes, designated C33, R2, and IA4 by different laboratories (*10*–*12*). C33 is associated with the inhibition of virus-induced syncytium formation (*10*), R2 is strongly up-regulated in mitogen-activated human T cells (*11*), and IA4 is expressed in large amounts in several B lymphocyte lines (*12*). One of these studies showed that the protein is localized to the cellular membrane and is highly glycosylated (*10*), which is consistent with the predicted molecular features.

To investigate whether *KAI1* was responsible for the metastasis suppression in AT6.1-11-1, we subcloned *KAI1* cDNA into a constitutive expression vector (*13*) and transfected it into parental AT6.1 cells. Individual transfectants were analyzed for *KAI1* expression and for their ability to suppress lung metastases of AT6.1 cells in nude mice (*14*). The vector alone was also transfected as a negative control. Expression of *KAI1* resulted in a significant suppression of the number of lung metastases per mouse, but it did not affect the growth rate of the primary tumor. These findings were observed in different experiments with multiple clones; one such experiment is shown in Table 1. Although the parental AT6.1 cells yielded 58 metastases per mouse, two transfectants that expressed large amounts of *KAI1* mRNA (similar to those for AT6.1-11-1) yielded only 6 or 7 metastases per mouse. Three vector control transfectants produced 30 to 47 metastases per mouse. Northern blot analysis showed that *KAI1* expression was undetectable or very low in 28 lung metastases from the *KAI1* transfectants, suggesting that selection for cells with absent or reduced *KAI1* expression resulted in metastasis formation. In addition, *KAI1* expression was reduced in human cell lines derived from metastatic prostate tumors (*15*) as compared with its expression in normal prostate tissue (Fig. 2); this finding suggests a possible suppressive role for *KAI1* in the metastasis of human prostate cancer.

To rule out the possibility that metastasis suppression by *KAI1* is the result of an indirect immune mechanism, we performed

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