Guanidine hydrochloride blocks a critical step in the propagation of the prion-like determinant [*PSI*$^+$] of *Saccharomyces cerevisiae*

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The cytoplasmic heritable determinant [*PSI*$^+$] of the yeast *Saccharomyces cerevisiae* reflects the prion-like properties of the chromosome-encoded protein Sup35p. This protein is known to be an essential eukaryote polypeptide release factor, namely eRF3. In a [*PSI*$^+$] background, the prion conformer of Sup35p forms large oligomers, which results in the intracellular depletion of functional release factor and hence inefficient translation termination. We have investigated the process by which the [*PSI*$^+$] determinant can be efficiently eliminated from strains, by growth in the presence of the protein denaturant guanidine hydrochloride (GuHCl). Strains are ''cured'' of [*PSI*$^+$] by millimolar concentrations of GuHCl, well below that normally required for protein denaturation. Here we provide evidence indicating that the elimination of the [*PSI*$^+$] determinant is not derived from the direct dissolution of self-replicating [*PSI*$^+$] seeds by GuHCl. Although GuHCl does elicit a moderate stress response, the elimination of [*PSI*$^+$] is not enhanced by stress, and furthermore, exhibits an absolute requirement for continued cell division. We propose that GuHCl inhibits a critical event in the propagation of the prion conformer and demonstrate that the kinetics of curing by GuHCl fit a random segregation model whereby the heritable [*PSI*$^+$] element is diluted from a culture, after the total inhibition of prion replication by GuHCl.

curing | Sup35p | cytoplasmic determinant

Recent biochemical evidence (1–5) has supported the hypothesis that the [*PSI*$^+$] phenotype of *Saccharomyces cerevisiae* reflects the prion-like properties of the *SUP35* gene product (6, 7). The essential chromosome-encoded protein Sup35p is known to be one of two eukaryote polypeptide release factors, namely eRF3 (8, 9). Sup35p associates with Sup45p (eRF1) *in vivo* to mediate translation termination (8). *In vitro*, Sup35p forms highly ordered fibers, whose appearance resembles that of fibrils formed by other amyloidogenic polypeptides (3, 4). In a [*PSI*$^+$] background, most Sup35p exists as large aggregates, possibly reflecting the propensity of this protein to form amyloid fibrils *in vivo* (1, 2). This property manifests as an allosuppressor phenotype (i.e., translation termination inefficient) in [*PSI*$^+$] cells, presumably because the cell is depleted of functional termination factors (10, 11). In normal growth conditions, [*PSI*$^+$] strains are metastable, with a relatively low frequency of reversion to [*psi*$^-$] (12, 13). However, Tuite *et al.* (13) demonstrated that growth in media containing very low concentrations (1–5 mM) of guanidine hydrochloride (GuHCl) converted yeast cells with up to 100% efficiency from [*PSI*$^+$] to [*psi*$^-$]. Similar observations were made for [*URE3*], a second prion determinant of *S. cerevisiae* (7). Other reagents including methanol, ethylene glycol, and hypertonic conditions, have been reported to exhibit curing properties; however, none of these cure with the near-total efficiency of GuHCl (13, 14).

Two hypotheses have been proposed to account for the curing properties of GuHCl. First, the elimination of the prion might arise directly from the ability of GuHCl to denature proteins. However, the concentrations of GuHCl effective in curing [*PSI*$^+$] are in the millimolar range, rather than the molar range typically required for the denaturation of proteins *in vitro* (13). Alternatively, GuHCl may actually promote the expression of an ancillary factor, namely the stress protein Hsp104p, which indirectly results in the reactivation of Sup35p and consequently the loss of the prion (15). Unlike other heat shock proteins, Hsp104p does not act to protect proteins against stress (i.e., heat denaturation), rather Hsp104p actively promotes the recovery of stress-denatured aggregated proteins by facilitating their refolding back into functional, native conformations (16, 17). Overexpression of Hsp104p might lead to the total refolding of Sup35p from the aberrant prion conformation to its native structure, thereby mediating prion loss. To test both hypotheses, we have examined the kinetics of prion elimination upon growth in the presence of GuHCl and assessed the influence of stress on the curing process.

Materials and Methods

Strain. The genotype of the strain used in this study was BSC783/4a: [*PSI*$^+$], *SUQ5, ade2–1, ura3–1, his3–11, his3–15, leu2–3, leu2–112, MATa.*

Growth Media. BSC783/4a was grown at 30°C on ¼YEPD solid medium [4% (wt/vol) glucose, 1% (wt/vol) Bacto-peptone, 0.25% (wt/vol) yeast extract, 2% (wt/vol) agar]. Most liquid cultures also were grown at 30°C in YEPD complete medium [2% (wt/vol) glucose, 1% (wt/vol) Bacto-peptone, 1% (wt/vol) yeast extract], with or without 3 mM GuHCl. For studies using ethanol-supplemented media, strains were grown in flasks, sealed with rubber bungs, to reduce evaporation of the alcohol from the medium.

Mathematical Expression of a Segregational Model. The line of best fit to a simple segregation model for curing was calculated as follows. A simple iterative computer model was generated where an initial population of cells, each with n [*PSI*$^+$] seeds, was followed through successive generations. For each generation the percentage of cells with n, n-1, n-2, . . . 2,1,0 prion seeds was calculated, assuming a simple random distribution of seeds between parent and daughter cells (i.e., the average number of [*PSI*$^+$] seeds per cell decreases 2-fold with each generation). Because the phenotype is assumed lost only when a cell is completely free of prion seeds, a plot of generation vs. percentage of cells with 0 prion seeds (i.e., % [*psi*$^-$]) gives a plot of curing for any initial value of n. However, such a model only generates a plot for integral numbers of generations. It is possible to generate an estimate for fractional generations by looking at variable values for n (where $n > 16$). For example, the plot for $n = 16$ is to a first approximation the same as the plot for $n =$

Abbreviation: GuHCl, guanidine hydrochloride.

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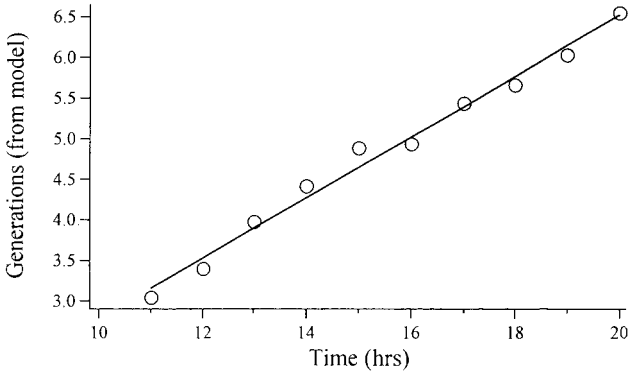

Fig. 1. Linear plot of the number of generations against growth time for one set of curing data in the presence of 3 mM GuHCl. The number of generations was obtained by comparing those values of % [*PSI*$^+$] determined experimentally at each time point, with those calculated from an iterative model for curing by random segregation, in which the initial number of prions (*n*) was set at 32. Only values of % [*PSI*$^+$] between 98 and 2 were considered. The line of best fit gives values for the gradient of 0.373 and the intercept of −0.934, with $R^2 = 0.982$. The gradient represents 1/generation time, yielding a value for calculated generation time of 2.68 hr. This calculation compares well with the experimentally determined generation time of 2.5 hr. The intercept gives a measure of the generation shift from the real number of prion seeds to that used in the iterative model. The best-fit number of prion seeds can be calculated by 32*(2-generation shift), yielding a value of 61. Similar linear plots for other curing curves in each case yielded generation times within 10% of the experimentally determined doubling time and an average number of prion seeds of 62 ± 10.

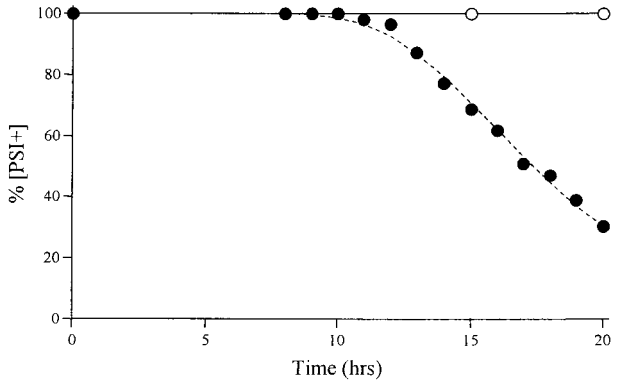

Fig. 2. Elimination of the [*PSI*$^+$] determinant by GuHCl requires cell division. Two identical cultures of the [*PSI*$^+$] strain BSC783/4a were grown at 30°C in YEPD supplemented with 3 mM GuHCl. One culture was maintained in exponential growth (●), while the other was allowed to enter stationary phase (○), after 10 hr (approximately four generations) of exponential growth. The [*PSI*$^+$] fraction of the cultures were determined, as a function of time, on the basis of the red/white colony ratio of culture aliquots, grown on solid ¼YEPD medium. Each data point represents an average of three culture samples. The dashed line represents the best fit to a simple segregational model, with variables pre-existing prion seeds and generation time (as described in *Materials and Methods*).

32, but shifted by one generation. Hence, the plot for $n = 28$ is effectively the plot for $n = 32$, shifted by 0.19 generations. Thus, a curve may be generated for $n = 32$ (by using integral values of $n = 16$–32), which has both integral and fractional values for generation number. A line of best fit may be generated for each of the curing data sets, by comparing the values of the experimentally determined % [*psi*$^-$] with those values derived from the model. A plot of the corresponding generation number (from the model) and time (from the experiment) gives a straight-line plot (Fig. 1). The gradient of this plot yields a measure of the generation time and the intercept gives a measure of the shift in generations required, from the experimental value for *n* to the model value of $n = 32$. For each set of experimental data, only values in the range between 2% and 98% [*psi*$^-$] were used for the best fit. The line of best fit for each set of data gave a calculated generation time that was within 10% of that determined experimentally.

Results

Exponential Growth Is a Prerequisite for Curing by GuHCl. To study the profile of [*PSI*$^+$] elimination from *S. cerevisiae* by GuHCl, the [*PSI*$^+$] variant of strain BSC783/4a was grown at 30°C in rich YEPD medium supplemented with 3 mM GuHCl. Two identical cultures were developed, one of which was allowed to enter stationary phase, after a short period of exponential growth, encompassing approximately four doubling times (i.e., ≈10 hr). The other culture was grown in identical medium, but was diluted into fresh YEPD + GuHCl as required (i.e., when the OD_{600} reached 0.8, a density of approximately 6×10^6 cells·ml^{-1}) to ensure continued exponential growth. During the course of the experiment, culture aliquots were removed at hourly intervals and the cells were harvested. The cells were washed with sterile water and diluted, and aliquots were spread onto ¼YEPD solid medium (typically 100–300 colony-forming units per plate), to determine the ratio of [*PSI*$^+$] and [*psi*$^-$] cells in the culture. The composition of the culture was determined on the basis of a white/red colony ratio, by virtue of the *ade2–1/SUQ5* marker system (18). Sectored colonies were scored according to the simplest model of segregation (i.e., a half-white, half-red colony was scored as two individual cells, one [*PSI*$^+$] [white] and one [*psi*$^-$] [red]).

As seen in Fig. 2, GuHCl was only able to elicit prion loss from a [*PSI*$^+$] culture that had sustained continued exponential growth. Intriguingly, the profile of prion elimination exhibited a significant lag, corresponding to approximately four generations, before the gradual emergence of [*psi*$^-$] (i.e., prion-free) cells in the exponential growth culture. The data infer that cell division is a prerequisite for curing by GuHCl. However, it is possible that the stress response associated with stationary phase might afford protection to the [*PSI*$^+$] determinant from the curing effects of GuHCl. A component of this stress response is the enhanced expression of Hsp104p and Hsp70p. In particular, the coexpression of Ssa1, a member of the Hsp70 family, has been shown to negate the curing effect of elevated Hsp104p levels on aggregates of Sup35p (19).

Stress Does Not Enhance Curing by GuHCl. To assess the influence of stress on curing, we studied the loss of the [*PSI*$^+$] determinant from strain BSC783/4a, when grown in media containing 3 mM GuHCl, with and without 3% (vol/vol) ethanol (Fig. 3). Ethanol induces both thermotolerance and the synthesis of heat shock proteins in *S. cerevisiae* (20), and growth for 1 hr in 3% (vol/vol) ethanol results in a clear increase in acquired thermotolerance in strain BSC783/4a (S.S.E. and M.F.T., unpublished data). The presence of 3 mM GuHCl had a modest effect on the growth of the strain in YEPD media, increasing the doubling time by approximately 10% to 2.5 hr. When grown in YEPD supplemented with both GuHCl and ethanol, the doubling time of the strain was increased approximately 2-fold to 5.5 hr (data not shown). The presence of 3% (vol/vol) EtOH in combination with 3 mM GuHCl would be expected to elicit a greater stress response than that induced by 3 mM GuHCl alone. Although the addition of ethanol clearly increased the stress upon the curing culture, as reflected by an increased doubling time, any corresponding induction of heat shock protein(s) did not enhance the rate of prion elimination (Fig. 3). Moreover, the time taken to

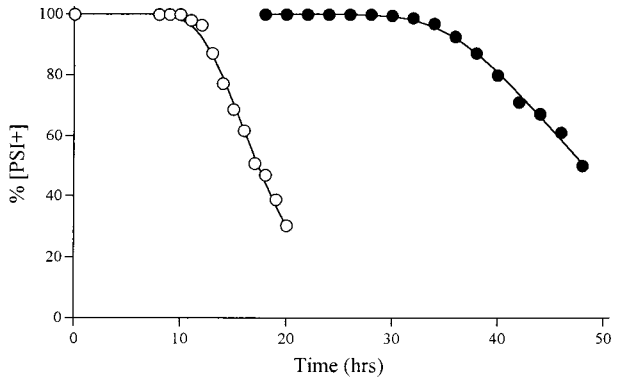

Fig. 3. Stress response does not enhance the rate of prion elimination by GuHCl. BSC783/4a [*PSI*$^+$] was grown in exponential phase at 30°C in YEPD containing 3 mM GuHCl (○) and further supplemented with 3% (vol/vol) EtOH (●). The [*PSI*$^+$] fraction of the cultures was determined, as a function of time, on the basis of the red/white colony ratio of culture samples. Each data point represents an average of three culture samples. The lines represent the best fit to a simple segregational model, with variables pre-existing prion seeds and generation time.

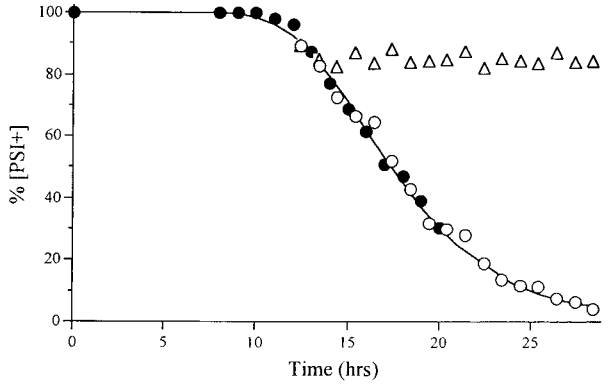

Fig. 4. The propagation of [*PSI*$^+$] is stable upon removal of cells from GuHCl. After 12.5 hr exponential growth in the presence of 3 mM GuHCl (●), two culture aliquots of BSC783/4a were transferred to fresh medium containing 3 mM GuHCl (○) or to YEPD medium alone (△). The cultures then were allowed to continue exponential growth. The [*PSI*$^+$] fraction of the cultures were determined, as a function of time, on the basis of the red/white colony ratio of culture samples. Each data point represents an average of three culture samples. The line overlaying the data for growth in the presence of GuHCl represents the best fit to a simple segregational model, with variables pre-existing prion seeds and generation time.

eliminate the [*PSI*$^+$] determinant was greatly increased by the presence of ethanol. It is noteworthy that no curing was observed in an identical culture of BSC783/4a grown in YEPD media supplemented with 3% (vol/vol) ethanol alone (data not shown). This observation highlighted the exceptional efficiency of GuHCl as a curing agent and demonstrated that stress per se, including prolonged growth at 37–39°C, is a relatively inefficient curing agent (refs. 13, 15, 20, and 21; S. Lindquist, personal communication).

GuHCl Ablates Propagation of the Prion Determinant. We propose that the profile of GuHCl-induced curing (Fig. 2) and the absolute requirement of cell division can best be explained by the hypothesis that GuHCl blocks a critical step in the replication of the prion conformer and that pre-existing prion seeds are "diluted" in cells during sustained exponential growth. Our hypothesis predicts that a [*PSI*$^+$] culture, grown in the presence of GuHCl, would display a segregational lag, before the emergence of prion-free cells (as seen in Fig. 2). During this lag, the average number of prion seeds within each cell is halved every doubling time, after the random segregation of any prion seeds between parent and daughter cell. The size of the segregational lag would depend on the average number of pre-existing seeds within a [*PSI*$^+$] cell, the doubling time of the culture, and the degree of inhibition of prion replication. This segregational model also predicts that it is possible to achieve the stable recovery of [*PSI*$^+$] cells at any stage during curing, although as time goes by the fraction of these become too few to detect.

To test this segregational hypothesis, two aliquots were withdrawn from a culture that had been growing for 12.5 hr (five generations) in the presence of GuHCl. The aliquots were either inoculated into fresh media containing GuHCl or "rescued" into YEPD media alone. As seen previously (Fig. 2), the [*PSI*$^+$] determinant was eliminated upon continued growth in the presence of GuHCl (Fig. 4). However, in agreement with our hypothesis, the rescued culture aliquot that was developed in the absence of GuHCl exhibited a stable fraction of [*PSI*$^+$] cells (Fig. 4).

Although the addition of 3% (vol/vol) EtOH did not reduce the time taken to eliminate the [*PSI*$^+$] determinant (Fig. 3), the data did support our segregational model. First, the presence of EtOH would be expected to accelerate curing, if the stress-induced expression of an ancillary factor, such as Hsp104p, was the means by which GuHCl induces the loss of the [*PSI*$^+$] determinant. This was not observed. Second, the GuHCl-induced curing profiles of the two cultures (in the presence or absence of EtOH) are identical, when the culture composition is analyzed with respect to the number of generations (Fig. 5, compare to Fig. 3). The elimination of [*PSI*$^+$] from a culture growing in YEPD medium supplemented with both GuHCl and EtOH takes much longer because the doubling time of the culture is increased. This, in turn, increases the time taken to segregate pre-existing seeds between parent and daughter cells, thereby making the dilution (curing) of [*PSI*$^+$] a slower process. The curves that fit the data points in Figs. 3–5 are theoretical, based on our hypothesis that the replication of [*PSI*$^+$] seeds is totally inhibited by GuHCl and that any pre-existing seeds then segregate at random between parent and daughter cells until [*psi*$^-$] (i.e., seed-free) cells emerge. The model permits a calcu-

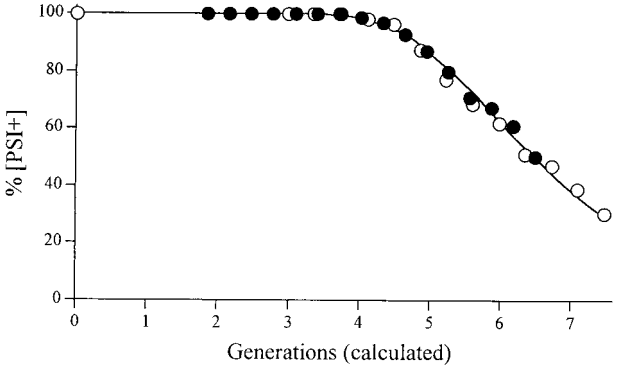

Fig. 5. Curing fits to a simple segregation model. The data of Fig. 3 are replotted with respect to generations, as calculated by the model and adjusted to account for a brief cessation of growth, during which the cells adapted to the presence of 3% (vol/vol) EtOH. BSC783/4a [*PSI*$^+$] was grown in exponential phase, at 30°C in YEPD containing 3 mM GuHCl (○) and further supplemented with 3% (vol/vol) EtOH (●). The curve is a theoretical plot, based on the segregational hypothesis, where the calculated number of pre-existing prion seeds within a cell is 62. The calculated generation times for both sets of data from the model were within 10% of the experimentally determined generation times.

lation of the average number of prion seeds per cell, at the time of addition of GuHCl, which is 62 ± 10. In addition to the average number of prion seeds per cell, the model generates an independent value for the generation time. The line of best fit for each set of data gave a calculated generation time that was within 10% of that determined experimentally.

Discussion

The [*PSI*$^+$] phenotype of *S. cerevisiae* reflects the prion-like behavior of the host-encoded protein Sup35p (eRF3). Sup35p does exhibit several properties of the mammalian prion protein (PrP) (reviewed in refs. 10 and 22). The prion-like properties of Sup35p can be eliminated by growth in the presence of millimolar concentrations of GuHCl (13). This chaotropic salt is widely used as a protein denaturant and indeed has even been shown to result in the loss of PrP infectivity when used at a concentration >3.5 M (23–25). The experimental data presented in this study do not support a model for curing, whereby GuHCl either directly or indirectly (through the induction of an ancillary factor) elicits the dissolution of any pre-existing prion seeds. We have shown that stress does not enhance curing in the presence of GuHCl. If the stress-induced expression of an ancillary factor (e.g., Hsp104p) were the underlying factor in [*PSI*$^+$] elimination by GuHCl, then curing might be expected to accelerate in stationary phase cultures or those grown in the presence of ethanol. The curing curves in the presence or absence of ethanol overlap when expressed in terms of generations (see Fig. 5). This observation is consistent only with a model of curing by inhibition of prion replication. However, this result does not indicate that the stress response is not involved in replication, only that it has no visible effect on curing. The data demonstrate that ethanol has no effect on curing by GuHCl and suggests that it is actually the inhibition of cell division that prevents elimination of the [*PSI*$^+$] determinant in stationary phase cultures.

The failure of GuHCl to eliminate the [*PSI*$^+$] determinant from a stationary phase culture (see Fig. 2) is not direct proof that growth is a prerequisite for curing. Newman *et al.* (19) have demonstrated the protective capacity of a stress response, and it could be that argued that such a mechanism protects the [*PSI*$^+$] determinant from the effects of GuHCl within stationary phase cells. However, the data shown in Fig. 5 implies that the stress response, known to be induced by ethanol (26, 27), either has no effect on curing (which it would not do in a segregation model if replication was completely inhibited) or that the presence of ethanol has a negative effect on curing that is precisely offset by its positive effect on the stress response (and any associated protection of Sup35p aggregates).

Curing of [*PSI*$^+$] by GuHCl exhibits an apparent requirement for growth and a segregational lag, which we propose reflects the particulate inheritance of [*PSI*$^+$] and its subsequent dilution from cells grown in the presence of GuHCl. Our data, under all conditions tested, fit extremely well to a random segregation model for curing, whereby there are a number of self-replicating particles within a [*PSI*$^+$] cell, which are randomly segregated between mother and daughter cells and whose propagation is inhibited by GuHCl. The data do not support a model of segregation in which the prion seeds were evenly or directionally segregated. For an equivalent number of prion seeds, a directional segregation model would be predicted to have a longer lag phase and a steeper gradient than that observed (i.e., the decrease from 100% to 50% [*PSI*$^+$] would be expected to occur within a single generation, under directional segregation).

The random segregation model for GuHCl-induced curing predicts that (*a*) growth is required, (*b*) a segregational lag would be observed before curing, and (*c*) stable recovery of [*PSI*$^+$] cells can be achieved at any stage during curing. All of these are observed experimentally. In addition, the independently calculated generation time from the model, for all data sets, was within 10% of that determined experimentally. This model is also consistent with a previous observation (28) that after prolonged treatment the absolute number of [*PSI*$^+$] colonies per unit volume reaches a plateau (i.e., GuHCl does not eliminate [*PSI*$^+$] determinants directly but stops their replication). It is noteworthy that high osmolarity has been reported to convert cells to [*psi*$^-$] directly (14), in contrast to the mechanism of GuHCl curing proposed here.

From our data, it is possible to calculate the number of self-replicating seeds within a [*PSI*$^+$] cell, which for strain BSC783/4a was approximately 60. A remarkably similar number has been deduced previously for two different [*PSI*$^+$] strains and by a completely different experimental approach (29). Several other conclusions can be drawn from the kinetic analysis. First, the shape of the curing curve and the fact that the calculated generation time is the same as that determined experimentally implies that GuHCl, at the concentration used, must completely inhibit the propagation of the prion seeds. Second, upon rescue stable recovery of [*PSI*$^+$] cells is achieved immediately, despite the low number of average prion seeds per cell. These findings suggest either that the segregation is not truly random (though it must appear so), or that replication of prion seeds once GuHCl is removed is extremely rapid in comparison to the generation time. Both of these also would be consistent with the very small number of sectored colonies observed in the *ade2–1/SUQ5* marker system.

As a provocative alternative to the prion hypothesis, the particulate pattern of [*PSI*$^+$] inheritance could be construed as evidence that [*PSI*$^+$] is in fact an autonomous nucleic acid, for example a virus or a plasmid. GuHCl is known to be a reversible inhibitor of poliovirus RNA replication at millimolar concentrations (30), to eliminate the penicillinase-encoding plasmid of *Staphylococcus aureus,* (31) and to efficiently induce nonchromosomal respiratory-deficient petites of *S. cerevisiae* with extremely high efficiency (32). GuHCl might plausibly elicit curing if it blocked the self-replication of a [*PSI*$^+$]-encoding nucleic acid species. However, unlike curing of [*PSI*$^+$], growth is not a prerequisite for petite induction in yeast (32) and despite the identification of many plasmids and viruses of yeast (33), no extrachromosomal nucleic acid has ever been linked to [*PSI*$^+$] (34, 35).

Perhaps the most compelling evidence that [*PSI*$^+$] is a protein-only phenomenon is the requirement of a specific level of Hsp104p for [*PSI*$^+$] maintenance (15). Originally, it was proposed that Hsp104p permits a conformational transition state in wild-type Sup35p, which facilitates its folding into a prion conformation (15). In an alternative "breathing template" model, Hsp104p might catalyze the partial denaturation of the prion conformer, thereby permitting an intermediate conformation that is required for the corruption of native protein (24, 25). However, the absolute requirement for Hsp104p in the conversion of wild-type protein to the prion conformer has been disputed, in light of both *in vitro* and *in vivo* data (3, 4, 36). Recently, it has been proposed that Hsp104p functions solely as a "disaggregase." Hsp104p is required to break up Sup35p polymers in a [*PSI*$^+$] strain, thereby promoting the continued inheritance of [*PSI*$^+$] seeds to all mitotic (and meiotic) progeny (2, 22, 37). The mechanism for generating new prion seeds has been termed secondary nucleation (38). Ironically, in fulfilling its normal cellular role as a disaggregase, Hsp104p actually may ensure the propagation of [*PSI*$^+$] in *S. cerevisiae*.

Because Hsp104p is a key factor in the replication of [*PSI*$^+$], it is plausible that GuHCl affects its activity directly, thereby resulting in the inhibition of prion replication. GuHCl could disrupt the action of Hsp104p, regardless of whether Hsp104p functions as a disaggregase to facilitate secondary nucleation, or by promoting the partial unfolding of the prion-like conformer of Sup35p, an event that may or may not be required for prion

propagation. Hsp104p bears two nucleotide-binding sites, the second of which is essential for the assembly of Hsp104p into a functional homohexameric complex (39, 40). Mutations within these ATP-binding sites have a profound effect on chaperone function, in terms of acquired thermotolerance (39), recovery of stress-denatured protein (16), and most intriguingly, in the maintenance of [*PSI*$^+$]. Expression of a mutant Hsp104p with both ATP-binding sites inactivated converted [*PSI*$^+$] cells to [*psi*$^-$] (1, 15). This double mutation of the *Hsp104* gene is a dominant *psi no more (PNM)* mutation (29), in that it causes the loss of [*PSI*$^+$] even in the presence of the chromosomal *HSP104* gene (15). The dominant effect of the double mutant is thought to arise from the oligomeric nature of functional Hsp104p. The coexpression of wild-type and mutant protein presumably gives rise to heterogeneous hexamers, which are defective for nucleotide hydrolysis and hence activity (15).

It is plausible that GuHCl has an adverse effect on the ATPase activity of Hsp104p, resulting in a loss of function and ultimately, the loss of the prion. Glover and Lindquist (17) have demonstrated that the ATPase activity of Hsp104p is extremely sensitive to low concentrations of GuHCl *in vitro*, although Hsp104p did not exhibit the same sensitivity to urea. Similarly, the transcriptional activity of T7 RNA polymerase is enhanced nearly 2-fold in the presence of 50 mM GuHCl and yet only exhibits denaturation and a loss of activity in the presence of urea (41). Although mM concentrations of GuHCl may not be sufficient to promote denaturation of proteins, it is clear that such levels can have a profound effect on protein activity. As an alternative, GuHCl might actually act on Sup35p itself. The low concentration of GuHCl could result in the stabilization of either the native or the prion conformer, thereby precluding partial denaturation and hence blocking prion replication. Such a mechanism of action has been proposed for the anion Congo red, which inhibits the corruption of native prion protein to the prion conformer (42). Either inhibition of Hsp104p or direct interaction with Sup35p preventing replication would be fully consistent with our random segregation model for GuHCl curing.

Although our data have not identified the biochemical means by which GuHCl is able to cure *S. cerevisiae* of the prion-like determinant [*PSI*$^+$], it has formed the framework for a random segregation model that describes the mechanism of curing. The model postulates that GuHCl inhibits a critical step in the replication of the prion conformer, whereby the heritable [*PSI*$^+$] determinant is diluted from a dividing culture, after the total inhibition of replication by GuHCl.

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