A protein required for prion generation: [URE3] induction requires the Ras-regulated Mks1 protein

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Infectious proteins (prions) can arise *de novo* as well as by transmission from another individual. *De novo* prion generation is believed responsible for most cases of Creutzfeldt–Jakob disease and for initiating the mad cow disease epidemic. However, the cellular components needed for prion generation have not been identified in any system. The [URE3] prion of *Saccharomyces cerevisiae* is an infectious form of Ure2p, apparently a self-propagating amyloid. We now demonstrate a protein required for *de novo* prion generation. Mks1p negatively regulates Ure2p and is itself negatively regulated by the presence of ammonia and by the Ras–cAMP pathway. We find that in *mks1*Δ strains, *de novo* generation of the [URE3] prion is blocked, although [URE3] introduced from another strain is expressed and propagates stably. Ras2^{Val19} increases cAMP production and also blocks [URE3] generation. These results emphasize the distinction between prion generation and propagation, and they show that cellular regulatory mechanisms can critically affect prion generation.

The word "prion" means infectious protein, a concept that arose in studies of mammalian transmissible spongiform encephalopathies, including Creutzfeldt–Jakob disease (CJD) of humans, scrapie of sheep, and bovine spongiform encephalopathy (BSE) (1–3). Although some CJD cases are due to mutations in the *Prnp* gene encoding PrP (prion protein), and over 50 cases were due to transmission of BSE material to humans, most cases are believed to be caused by *de novo* prion generation. *De novo* prions may also be the original source of BSE. Attempts to study prion initiation or generation in animal systems have met little success, in part because such events are rare, and most studies have been restricted to examination of the mechanism of propagation.

The nonchromosomal yeast genetic elements [URE3] (4) and [PSI+] (5) were identified (6) as infectious protein (prion) forms of Ure2p and Sup35p, respectively, based on their genetic properties being inconsistent with a nucleic acid replicon but exactly what one would expect of a prion. First, if a prion can be cured, it will arise again in the cured strain at some low frequency because the normal form of the protein is still present. Second, overproduction of the protein will increase the frequency of *de novo* prion appearance. Third, the gene for the protein is needed for prion propagation, but the phenotype of mutants in the gene will be the same as the phenotype produced by the presence of the prion—because in both cases the normal form is deficient. In fact, [URE3] and [PSI+] satisfy these criteria as prions of Ure2p and Sup35p, respectively (6). In particular, *de novo* appearance of [URE3] is induced by overproduction of Ure2p (6–8) and appearance of [PSI] is induced by overproduction of Sup35p (9, 10).

Amyloid is a special form of protein characterized by filamentous morphology, high β-sheet content, protease resistance, and yellow-green birefringence on staining with the dye Congo red (reviewed in ref. 11). Amyloid accumulation is a prominent feature of Alzheimer's disease, late-onset diabetes mellitus, CJD, and many other conditions. Biochemical and cell biological studies suggest that amyloid formation by Ure2p and Sup35p is the basis for the [URE3] and [PSI+] prions (7, 12–18). In extracts of [URE3] strains, Ure2p is partially protease resistant (7), and Ure2p-green fluorescent protein (GFP) fusion proteins are aggregated *in vivo* specifically in [URE3] cells (17). Ure2p can form amyloid *in vitro*, a reaction that is specifically promoted by the part of the Ure2p molecule responsible for prion formation *in vivo* (18). The pattern of protease-resistant fragments found with Ure2p amyloid formed *in vitro* (18) is the same as that seen in extracts of [URE3] strains (7). These results suggest that [URE3] is an infectious amyloidosis of Ure2p.

Ure2p plays a central role in the control of nitrogen catabolism of yeast. When cells are presented with a good nitrogen source (such as ammonia), Ure2p turns off transcription of genes needed for using poor nitrogen sources (such as allantoate) by blocking the action of the positive transcription factor Gln3p (4, 19–25). Dal5p, the allantoate permease, is controlled by Gln3p and can also take up ureidosuccinate (USA), an essential intermediate in uracil biosynthesis. Thus, the uptake of USA by Dal5p can be used as an assay of this nitrogen catabolite repression (4, 19, 26). Either *ure2* mutants or cells with the [URE3] prion will take up USA even on media containing the good nitrogen source ammonia, whereas wild-type cells will not.

Mks1p was originally described as an inhibitor of growth whose action is blocked by activity of the Ras–cAMP pathway (27). For example, deletion of *MKS1* partially relieves the effects of deficiency of cAMP, suggesting that antagonizing the negative action of Mks1p is a major task of the cAMP pathway. Recently, we reported that Mks1p is part of the nitrogen regulation cascade (28) as follows: $NH_3 \dashv$ Mks1p $\dashv$ Ure2p $\dashv$ Gln3p $\rightarrow$ *DAL5*. Mks1p blocks the action of Ure2p unless it receives a signal indicating that a good nitrogen source (such as ammonia) is present. Thus, in the absence of Mks1p, *DAL5* transcription is not activated on a poor nitrogen source. Furthermore, overexpression of Mks1p inhibits Ure2p action even in the presence of ammonia, but does not alter the steady-state levels of Ure2p (28).

In this paper we find that deletion of *MKS1* dramatically impairs ability of Ure2p to change into the [URE3] prion form, whereas overproduction of Mks1p modestly increases the frequency of this prion change. Until now, there was no indication of a connection between the Ras–cAMP pathway and any prions. However, the fact that the Ras–cAMP pathway inhibits Mks1p (27) suggested to us that a constitutive *RAS* allele would

Abbreviations: CJD, Creutzfeldt–Jakob disease; BSE, bovine spongiform encephalopathy; GFP, green fluorescent protein; PrP, the mammalian prion protein; USA, ureidosuccinate; [URE3], the presence of the prion form of Ure2p; [ure-o], the absence of the [URE3] prion; $\dashv$, inhibits or negatively regulates; $\rightarrow$, stimulates or positively regulates.

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likewise impair [URE3] prion generation, and we find this to be the case.

Materials and Methods

Strains and Media. Wild-type strain YHE711 (*MATα ura2 leu2Δ::hisG*) and *mks1Δ* strain YHE710 (*MATα ura2 leu2Δ::hisG mks1Δ::*G418 [ure-o]), both in the Σ1278 background, have been described (28). Strains YHE869 (*MATα ura2 leu2Δ::hisG trp1Δ::hisG* [ure-o]) and YHE879 (*MATα ura2 leu2Δ::hisG trp1Δ::hisG mks1Δ::*G418) also have the Σ1278 background. Rich medium (YPAD, yeast extract/peptone/adenine/dextrose), minimal ammonium/dextrose medium (SD), minimal galactose raffinose medium (SGalRaf), and synthetic complete medium (SC) have been described (29). USA was added at 100 μg/ml as indicated.

Plasmids. pH7 and pH14 were described (17). The *ADH1* cassette from pVT103 (30) amplified with oligonucleotides HE66 and HE67 (31) was inserted into *Pvu*II-digested pRS425 (32) with opposite orientation to *LEU2,* creating pH125. Oligonucleotide HE93 (5′-AATTGCTAGC-3′) was ligated into the *Eco*RI site of the *GAL1,10* promoter from pBM272 (33), and the *Nhe*I–*Bam*HI-bordered *GAL1,10* promoter was used to replace the similarly bordered *ADH1* promoter of pH125, creating pH317. The *URE2* ORF was cloned as a *Bam*HI/*Xho*I fragment from pH14 into the same window of pH317 producing pH376. *URE2ΔApa*I, deleted for 24 bp around the *Apa*I site in the C-terminal part of the *URE2* ORF (7), was transferred from p725 into the *Bam*HI/*Xho*I window of pH317, forming pH377. The part of *URE2* encoding residues 1–89 was transferred from X4C (34) into the *Bam*HI/*Xho*I window of pH317, forming pH381. The *Not*I and *Xho*I sites of pVTG20 (17) were fused, introducing a stop codon at position 66 by using oligonucleotides HE94 (GGCCGCTAAGAGC) and HE95 (TCGAGCTCTTAGC) and the resulting $URE2^{1-65}$ fragment was cloned into the *Bam*HI/*Xho*I window of pH317, forming pH382. *URE2ΔApa*I was transferred from p725 into the *Bam*HI/*Xho*I window of pH7, forming pH438.

The *MKS1* promoter was amplified by PCR from strain Σ1278b by using oligonucleotides HE152 (AAAGCTAGCTC-TATCTATAAGGACATTCC) and HE153. (AAAGGATC-CATTTGAGAATAATTAGGAAGTG). The resulting PCR product was used to replace the *Nhe*I–*Bam*HI-bordered *ADH1* promoter in pH122 [pRS314 (35) in which the *ADH1* cassette from pVT103 (30, 31) was inserted into the *Pvu*II window in the opposite direction to *TRP1*], forming pH417. The full-length *MKS1* ORF was cloned as a *Bam*HI/*Xho*I fragment from pH230 (28) into the same window of pH417, forming pH431. pH341 is pRS314 in which the *Hin*dIII and *Xba*I sites have been removed from the *TRP1* gene by using oligonucleotides HE128 (AA-GAGAGCCCCGAAAGTTTACATTTTATGTTAGCTG) and HE129 (GGCCGCAGAATGTGCTCTTGATTCCGAT-GCTGACTTG), respectively. $Ras2^{Val19}$ was cloned as a *Eco*RI/*Hin*dIII fragment from YCpR2V (M. Wigler, generous gift from G. Fink) into the same window of pH341, forming pH406.

[URE3] Prion Induction *de Novo*. [ure-o] strains were transformed with vector control or a plasmid overproducing Ure2p or a fragment thereof. Transformant colonies were individually grown to saturation in medium selective for the plasmid. Starting from 10^7 cells per plate, serial 10-fold dilutions were plated on SD + USA. Colonies appearing after 5 days at 30°C were recorded.

Cytoduction. Transfer of cytoplasm from cell to cell without nuclear fusion was carried out with the *kar1-1* mutant (36), in which nuclear fusion is defective. The recipient was made ρ^o by ethidium treatment, whereas the donor was ρ^+. Donor and recipient cells of opposite mating type, one of which is *kar1-1,* were mixed and allowed to mate for 6–8 h. Cells were then plated on media selecting against the donor, and ρ^+ colonies with the nuclear genotype of the recipient were the cytoductants (37).

Table 1. [URE3] generation is reduced in *mks1Δ* cells

Host	Plasmid	USA^+ per 10^6 cells
	A. Constitutive Ure2p overexpression	
Wild type	pH7 P_{ADH1}	3
	pH14 P_{ADH1}*URE2*	240
*mks1Δ::*G418	pH7 P_{ADH1}	<0.1
	pH14 P_{ADH1}*URE2*	<0.1
	B. Transient Ure2p overexpression	
Wild type	pH317 P_{GAL1}	4
	pH376 P_{GAL1}*URE2*	210
	pH377 P_{GAL1}*URE2ΔApaI*	15,000
	pH381 $P_{GAL1}URE2^{1-89}$	410
	pH382 $P_{GAL1}URE2^{1-65}$	610
*mks1Δ::*G418	pH317 P_{GAL1}	<0.1
	pH376 P_{GAL1}*URE2*	0.2
	pH377 P_{GAL1}*URE2ΔApaI*	76
	pH381 $P_{GAL1}URE2^{1-89}$	<0.1
	pH382 $P_{GAL1}URE2^{1-89}$	<0.1
	C. *MKS1* corrects [URE3] induction defect of *mks1Δ* mutant	
*mks1Δ::*G418	pH417 vector + pH7 vector	2
	pH417 vector + pH14 P_{ADH1}*URE2*	0
	pH417 vector + pH438 P_{ADH1}*URE2ΔApaI*	10
	pH431 *MKS1* + pH7 vector	3
	pH431 *MKS1* + pH14 P_{ADH1}*URE2*	48
	pH431 *MKS1* + pH438 P_{ADH1}*URE2ΔApaI*	20,500

The wild-type (YHE711 = *MATα ura2 leu2Δ::hisG*) and *mks1Δ* (YHE710 = *MATα ura2 leu2Δ::hisG mks1Δ::*G418) strains were in the Σ1278 background (28). In part A, two transformants were grown to stationary phase in SC − Leu medium and dilutions were plated on SD + USA (100 μg/ml). In part, B, three transformants for each construct were grown to stationary phase in SGalRaf medium and dilutions were plated on SD + USA. In part C, strain YHE879 (*MATα ura2 leu2Δ::hisG trp1Δ::hisG mks1Δ::*G418), also having the Σ1278 background, was transformed with plasmids: pH417 = CEN *TRP1* P_{MKS1}, pH431 = CEN *TRP1* P_{MKS1}*MKS1*, pH7 = 2 μ *LEU2* P_{ADH1}, pH14 = 2 μ *LEU2* P_{ADH1}*URE2*, pH438 = 2 μ *LEU2* P_{ADH1}*URE2ΔApaI. URE2ΔApaI* lacks 8 amino acids in the C-terminal part of Ure2p, resulting in a 100-fold increase in the frequency with which [URE3] appears *de novo* (7). Three transformants for each construct were analyzed. The average number of colonies formed after 5 days of incubation at 30°C is shown. Colonies formed per 100 cells plated were 62–72 in A and 18–64 in B.

Results

***mks1Δ* Cells Are Defective in Generating [URE3].** Because Mks1p interacts functionally with Ure2p in nitrogen regulation, we examined [URE3] generation and propagation in cells in which Mks1p was deleted. The frequency of *de novo* formation of [URE3] in an *mks1Δ* strain was markedly decreased, whether we measured the rate of [URE3] formation in cells with the normal amount of Ure2p or that induced by overproduction of Ure2p from the constitutive *ADH1* promoter (Table 1 part A). The *mks1Δ* mutation also dramatically impaired the *de novo* induction of [URE3] detected on glucose media after transient overproduction of Ure2p, prion domain fragments, or deletion derivatives of Ure2p using the *GAL1* promoter (Table 1, part B). Controls showed that this was not because of decreased viability of the *mks1Δ* cells. Nor was the expression or overexpression of Ure2p affected by the *mks1Δ* mutation (Fig. 1*A*). The migration of Ure2p on SDS gels or isoelectric focusing gels was also unaffected by the *mks1Δ* mutation (Fig. 1 *A* and *B*). Complementation of the *mks1Δ* mutation by the *MKS1* gene on a single-copy plasmid restored ability to induce [URE3], confirm-

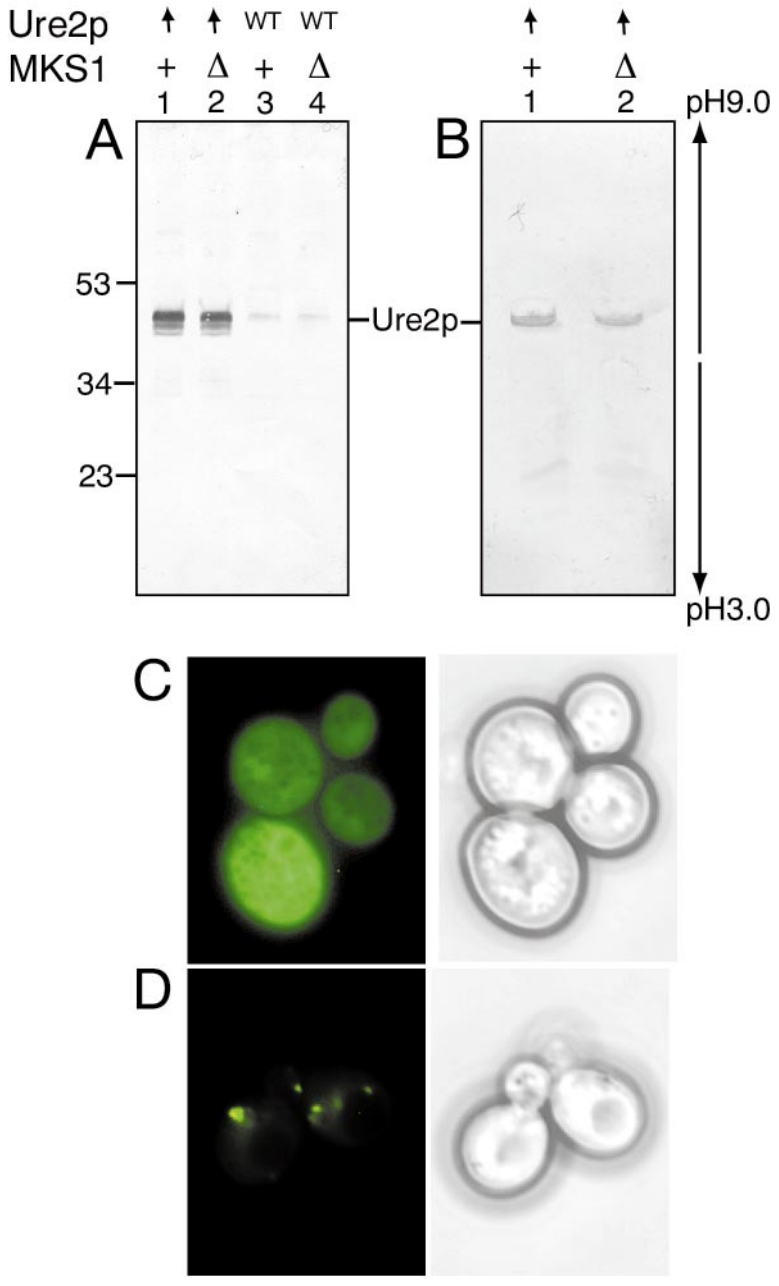

Fig. 1. Ure2p levels and distribution are not changed in *mks1*Δ cells. Strains YHE710 (*ura2 leu2*Δ *mks1*Δ, lanes A2, A4, B2) and YHE711 (*ura2 leu2*Δ *MKS1*, lanes A1, A3, B1) containing pH7 (vector, lanes A3, A4) or pH14 (P_{ADH1}*URE2*, lanes A1, A2, B1, B2) were grown to logarithmic phase in leucine-dropout medium. Protein extracts were prepared, and 10 μg was electrophoresed on an SDS/10–20% polyacrylamide gel (*A*) or a pH 3–9 isoelectric focusing gel (Pharmacia) (*B*). After blotting onto a poly(vinylidene difluoride) (PVDF) membrane Ure2p was visualized by using Ure2 antibody #2 (6). (*C* and *D*) Distribution of Ure2p-GFP in YHE710 (*mks1*Δ [ure-o]) (*C*) or YHE730 (*mks1*Δ [URE3]) (*D*) cells. Cells were transformed with pVTG12 (CEN *LEU2* P_{URE2}*URE2*$^{1-65}$-GFP). The images on the left show fluorescence from GFP, and those on the right are the phase-contrast photos of the same cells. Microscopy was performed as previously described (31).

ing that this difference was due to the *MKS1* gene (Table 1, part C). Because fragments of Ure2p are far more effective at inducing [URE3] appearance than is the intact protein (7), we considered the possibility that failure of degradation of Ure2p in an *mks1*Δ strain might limit prion induction. Although we cannot entirely rule out this possibility, there is no apparent difference in Ure2p degradation in wild-type and *mks1*Δ strains (Fig. 1 *A* and *B*). Moreover, the induction of [URE3] by fragments of Ure2p is as defective as that by the intact protein (Table 1).

mks1*Δ Cells Stably Propagate [URE3].** The inability of [URE3] to arise *de novo* in *mks1*Δ strains could be explained if [URE3] could not propagate in such cells. To test this possibility, [URE3] was transferred by cytoduction from YHE256 (*MATa** *ura2 arg*$^-$ [URE3]) to strains YHE710ρ^o (*MAT*α *ura2 leu2*::*hisG mks1*Δ::G418) and YHE711ρ^o (*MAT*α *leu2*::*hisG ura2 MKS1*). For each cytoduction all of 36 diploids analyzed could grow on ammonium/USA plates. All 22 cytoductants of strain YHE711 (*MKS1*) and all 16 cytoductants of strain YHE710 (*mks1*Δ::G418) could grow on ammonium/USA plates, indicating that *mks1*Δ cells could propagate [URE3]. To confirm that these USA$^+$ *mks1*Δ and *MKS1* cells were really carrying [URE3], three independent isolates of each were used as cytoduction donors to strain 3347 (*MAT***a** *kar1-1 ura2 arg*$^-$ [ure-o]). All of the 36 cytoductants that received cyto-

Table 2. Guanidine curing of [URE3] from *mks1*Δ and wild type

USA$^+$ colony	No. of USA$^+$ subclones			
	0 mM	1 mM	2 mM	3 mM
MKS1 1	18	18	0	0
2	18	18	0	0
3	18	6	0	0
4	18	13	0	0
*mks1*Δ 1	18	5	0	0
2	13	15	0	0
3	7	15	0	0
4	18	18	0	0

USA$^+$ colonies of *mks1*Δ (YHE730) and *MKS1* (YHE731) strains, produced by cytoduction (see text) were streaked to single colonies on YPAD plates containing 0 mM, 1 mM, 2 mM, or 3 mM guanidine·HCl. Eighteen single colonies from each treatment were placed in a grid on YPAD plates and replicated onto ammonium/USA plates. The number of USA$^+$ colonies is given.

plasm from the *MKS1* USA$^+$ strains and all of the 23 cytoductants that received cytoplasm from the *mks1*Δ USA$^+$ strains could grow on ammonium/USA plates. Thus an *MKS1* deletion does not prevent propagation of [URE3].

The stability of [URE3] in the *mks1*Δ strain was examined by testing clones grown in rich medium without selection (Table 2). [URE3] was only slightly less stable in the *mks1*Δ strain than in the wild type, a difference insufficient to account for the dramatic difference in the appearance of *de novo* [URE3] clones. [URE3] curing by guanidine was also similar from the *mks1*Δ and *MKS1* strains (Table 2).

Ure2p is evenly distributed in [ure-o] cells, but aggregated in [URE3] cells (17). This pattern is unchanged in *mks1*Δ cells, as shown by the distribution of a Ure2p$^{1-65}$-GFP fusion protein (Fig. 1 *C* and *D*). Mks1p does not affect the size or number of aggregates per cell.

Overexpression of Mks1p Increases *de Novo* Prion Appearance. High overexpression of Mks1p made cells USA$^+$, but on loss of the plasmid this phenotype disappeared, indicating that the effect was due not to appearance of [URE3] but to a negative regulatory effect of Mks1p on Ure2p (28). To avoid this problem and look for a smaller effect, we introduced *MKS1* on a single-copy vector with the native *MKS1* promoter (Table 3). We found a consistent elevation of [URE3] frequency. Similar results were obtained by using Mks1p transiently overexpressed from the *GAL1* promoter or with both Mks1p and Ure2p or its prion domain each expressed from a *GAL1* promoter (data not shown). Thus, strong overexpression of Mks1p results in growth

Table 3. Increase in Mks1p expression increases [URE3] incidence

Plasmids			USA$^+$ per 10^6 cells
CEN P_{MKS1}	+	2 μ P_{ADH1}	2
CEN P_{MKS1}	+	2 μ P_{ADH1}*URE2*	30
CEN P_{MKS1}	+	2 μ P_{ADH1}*URE2*Δ*ApaI*	1,200
CEN P_{MKS1}*MKS1*	+	2 μ P_{ADH1}	11
CEN P_{MKS1}*MKS1*	+	2 μ P_{ADH1}*URE2*	1,500
CEN P_{MKS1}*MKS1*	+	2 μ P_{ADH1}*URE2*Δ*ApaI*	32,000

Single-copy plasmids expressing Mks1p from its native promoter were used to obtain a modest increase in expression of this protein. Transformants of YHE869 (*MATα ura2 leu2*Δ::*hisG trp1*Δ::*hisG* [ure-o]) with the indicated plasmids were grown without tryptophan or leucine and suspended in water, and dilutions were plated on SD + USA. Number of colonies formed after 5 days is shown. Plasmids: pH7 = 2 μ *LEU2* P_{ADH1}, pH14 = 2 μ *LEU2* P_{ADH1}*URE2*, pH438 = 2 μ *LEU2* P_{ADH1}*URE2*Δ*ApaI*, pH417 = CEN *TRP1* P_{MKS1}, pH431 = CEN *TRP1* P_{MKS1}*MKS1*. The experiment was carried out four times with similar results; typical data are shown.

Table 4. Activated Ras2 blocks [URE3] prion induction

Constructs	USA$^+$ per 10^6 cfu
Vector + vector	7
Vector + P_{ADH1}*URE2*	63
Vector + P_{ADH1}*URE2*Δ*ApaI*	31,000
RAS2^{Val19} + vector	0
RAS2^{Val19} + P_{ADH1}*URE2*	0
RAS2^{Val19} + P_{ADH1}*URE2*Δ*ApaI*	400

Strain YHE869 (*MATα ura2 leu2*Δ::*hisG trp1*Δ::*hisG* [ure-o]) was transformed with a centromeric *TRP1* vector (pH341) or the same vector containing the constitutive active *RAS2*^{Val19} allele (pH406). In addition, the strain was transformed with *URE2* expression plasmids (pH7, 2 μ *LEU2* P_{ADH1}, pH14, 2 μ *LEU2* P_{ADH1}*URE2*; pH438, 2 μ *LEU2* P_{ADH1}*URE2*Δ*ApaI*). Three individual transformants for each combination were grown to saturation in medium without tryptophan or leucine. [URE3] generation was assayed by ability to grow on ammonium/USA medium and corrected for colony-forming units (cfu) per 100 cells. For strains carrying pH341 there were on average 30 cfu per 100 calculated cells. For strains carrying pH406 there were on average 9 cfu per 100 calculated cells. The average number of colonies per 10^6 cfu after 5 days for the three transformants is given.

of all cells on USA independent of their carrying [URE3] or not, a regulatory effect that makes direct measurement of [URE3] frequency impossible. However, modest overexpression of Mks1p does not have this regulatory effect and shows a substantial increase in [URE3] generation frequency. In summary, prion generation goes down when Mks1p is eliminated and goes up when Mks1p is slightly overproduced.

Constitutive Ras Blocks [URE3] Prion Generation. Mks1p action is inhibited by the Ras–cAMP system (27), and we find that *mks1*Δ results in an inability to induce the [URE3] prion. This observation suggests that activation of the Ras–cAMP pathway might likewise block [URE3] induction by inhibiting Mks1p. Indeed, expression of the constitutively active dominant *Ras2*^{Val19} allele largely prevented induction of [URE3] by either the intact Ure2p or the hyperactive Ure2pΔ*Apa*I fragment (Table 4).

Discussion

Little is known about the mechanism of spontaneous formation of the mammalian transmissible spongiform encephalopathies. Although PrP can convert to amyloid and other forms rich in β-sheet structure *in vitro*, such structures have not yet been shown to initiate the infectious process. Proteins capable of forming amyloids *in vivo* have been shown to form amyloid *in vitro*, but only after prolonged incubation of pure solutions at high concentrations. In most cases, such high concentrations do not exist *in vivo*, and certainly not as pure solutions. Thus, cellular (or extracellular) components other than the amyloid-forming proteins could have pronounced effects on the initiation and propagation of prions and amyloids. The study of such rare events is impossible in animal systems, but entirely feasible in yeast.

Propagation of [PSI+] is controlled by levels of Hsp104, with either increased or decreased levels of this chaperone resulting in loss of the prion (38). Other chaperones appear to modulate these effects (39, 40). Prion generation is finely controlled by the structure of the protein that becomes the prion (7, 10, 34, 41, 42). There appear to be both prion-promoting and prion-inhibiting regions of Ure2p and Sup35p, and their complex interactions are only beginning to be dissected. Overproduction of Sup45p prevents generation of [PSI+] (43). Since Sup45p normally forms a heterodimer with Sup35p, free Sup35p is probably the precursor of the [PSI+] form of the protein.

We show here that Mks1p is necessary for [URE3] to arise *de novo*, either from basal Ure2p levels or when Ure2p or its fragments are overproduced. Surprisingly, Mks1p is not necessary for [URE3] propagation. This description of a protein that is necessary for prion induction but not for prion propagation indicates that these two processes can be regulated in fundamentally different ways. We previously showed that the nitrogen source regulates Ure2p activity through Mks1p (28). Formally, the presence of ammonia inhibits the ability of Mks1p to inhibit Ure2p. This observation suggests the possibility that ammonia might also affect prion induction by Ure2p. However, nitrogen source does not affect [URE3] generation (ref. 8 and unpublished data). This finding indicates that these two effects of Mks1p on Ure2p are distinct. Nitrogen repression and derepression are also each compatible with [URE3] propagation (8), again distinguishing nitrogen regulation and the prion phenomenon. Since prion propagation is primarily a function of the N-terminal 80 residues of Ure2p, whereas nitrogen regulation is carried out by the Ure2p C-terminal domain (7, 34), our results suggest that Mks1p affects both parts of the Ure2p molecule. We have not detected covalent modification of Ure2p by Mks1p (Fig. 1*B*), but the mechanism of the Mks1p effect on Ure2p remains unclear.

In their original description of *MKS1*, Matsuura and Anraku (27) suggested that the Ras–cAMP pathway inhibits Mks1p activity. Our finding that [URE3] *de novo* generation was markedly defective in *mks1*Δ strains leads to the prediction that the *Ras2*^{Val19} mutation, by inactivating Mks1p, would likewise result in decreased frequency of [URE3] generation. The verification of this prediction indicates that cellular regulatory mechanisms outside the nitrogen control cascade can influence [URE3] prion formation. The broader implication is that any prion is likely to be affected by a range of cellular factors, some acting through a cascade of effects.

Our finding that Mks1p is necessary for generation of [URE3] could mean that an interaction of Ure2p and Mks1p is necessary for [URE3] prion formation. Alternatively, Mks1p may act through other cell components to allow conversion of Ure2p to the [URE3] prion form. Once conversion into the prion form is initiated, its propagation is not influenced by Mks1p, suggesting that the energy barrier for initiation is higher than that for propagation. It is likely that proteins interacting with PrP will similarly affect the frequency of spontaneous CJD. Serum amyloid P, basement membrane components (including glycosaminoglycans), and apolipoprotein E (apoE) are all regularly found in amyloid deposits (11). The association of the apolipoprotein E4 allele with Alzheimer's disease and the slowed development of amyloid A in apoE-knockout mice (44, 45) may reflect a relation similar to that we have found for Mks1p and the [URE3] amyloidosis of yeast.

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