8. The exponential loudness model for the 1000-Hz standard stimulus has been established in a previous study that used binaural loudness balance data between electric and acoustic stimulation in three brainstem implant subjects who had substantial hearing in their nonimplanted ears [F.-G. Zeng and R. V. Shannon, *Hear. Res.* **60**, 231 (1992)]. A similar finding was reported in two additional cochlear implant subjects [(*4*); M. F. Dorman *et al.*, *Ear Hear.* **14**, 290 (1993)].

9. An implant listener first listened to a pulsed stimulus train consisting of the 1000-Hz sinusoid standard. By pointing on a touch-sensitive tablet, the subject would hear a comparison sound alternating with the standard. The amplitude of the comparison sound was changed as the subject moved the pointing position up and down. The amplitude range on the touch-sensitive tablet was changed so that the absolute pointing did not indicate an absolute level. The consistency of the balance technique was indicated by the reproducibility of the measurement across sessions for some individual subjects and by the demonstration of transitivity among stimuli (for example, if A was balanced to B, and B was balanced to C, then A should be balanced to C). The comparison stimuli were sinusoids of 100 Hz, 300 Hz, and 3000 Hz, and biphasic pulse trains (100 μs per phase) of 100 Hz and 1000 Hz. All stimuli had a duration of 200 ms and a linear ramp of 5 ms. Stimuli were digitally generated through a 12-bit D/A converter at a sampling rate of 20 kHz (Data Translation DT2801-A) and controlled by a portable PC computer. Electric stimulation was delivered through an optically isolated constant-current source [L. S. Vurek *et al.*, *Ann. Otol. Rhinol. Laryngol.* **90** (suppl. 82), 21 (1981)]. Subjects were connected to the current source through a safety cutoff switch that allows a rapid disconnection from the stimulation setup in the event of experimenter error or hardware failure that might cause loud or unpleasant stimulation. In cochlear implants, the most apical electrode and monopolar stimulation were used. In brainstem implants, electrodes without nonauditory side effects were used.

10. The dynamic range was defined as the level difference between the absolute threshold and the uncomfortable loudness level (ULL), which were measured with a combination of Bekesy tracking and the method of limits (*15*). The threshold and the ULL (in microamperes) for each stimulus are represented by the two numbers in the parentheses following the subject's initials. For 100-Hz sinusoid: BO (0.8, 38), DC (1, 60), JB (0.7, 15), JP (1, 13), MK (3, 89), MM (1, 44), MP (1, 89), and RM (3, 38). For 300-Hz sinusoid: BO (5, 67), DC (10, 135), and MK (15, 112). For 1000-Hz sinusoid: BO (13, 106), DC (20, 200), JB (9, 141), JP (14, 75), MK (21, 167), MM (30, 180), MP (15, 224), and RM (24, 119). For 3000-Hz sinusoid: BO (20, 177), DC (35, 360), and MK (31, 112). For 100-Hz pulse: BO (80, 348), DC (120, 540), JB (97, 317), JP (85, 199), and KM (117, 488). For 1000-Hz pulse: BO (32, 224), DC (60, 440), JB (34, 313), JP (41, 189), KM (73, 357), and MP (33, 263). For three brainstem implant listeners, the threshold and the ULL are as follows. For 1000-Hz sinusoid: CB (150, 550), JP (60, 320), and KM (250, 600). For 100-Hz pulse: CB (310, 660), JP (220, 680), and KM (600, 950).

11. Only the 100-Hz pulse was tested because the high threshold for the 100-Hz sinusoid would have exceeded the safety level in brainstem implant subjects [R. V. Shannon, *IEEE Trans. Biomed. Eng.* **39**, 424 (1992)].

12. Neural synchrony or timing may be involved in determining the difference in loudness functions observed between the cochlear and brainstem implant subjects. Though the neural synchrony has been shown to occur for stimuli as high as 10 kHz in the auditory nerve, the usable range by the central auditory system may be limited to only 300 Hz. This is evidenced by both neural recording in the central auditory system, in which the synchronized response to modulation or signal frequen-

cies rarely exceeds 300 Hz [for example, A. R. Moller, *Brain Res.* **57**, 443 (1973); R. D. Frisina *et al.*, *Hear. Res.* **44**, 99 (1990); J. J. Eggermont, *ibid.* **56**, 153 (1991)] and by the measurement of temporal pitch with sinusoid-modulated noise in normal-hearing subjects [E. M. Burns and N. F. Viemeister, *J. Acoust. Soc. Am.* **60**, 963 (1976)] or in cochlear implant subjects (*15*). One possible temporal mechanism for the coding of loudness is that loudness is related to a measure of the synchronized rate for low-frequency stimuli and of the overall rate for high-frequency stimuli. This suggestion is based on the observation of the auditory nerve recording that the ratio of the synchronized rate to the overall rate appears to be a logarithmic function of the sound intensity [D. O. Kim and C. E. Molnar, *J. Neurophysiol.* **42**, 16 (1979)], analogous to the present logarithmic loudness balance function between low- and high-frequency electric stimuli.

13. In animals with acoustic hearing, the cochlear mechanics approaches linearity at the apex of the cochlea, a phenomenon that has been suggested to reflect a smaller degree of involvement of the active process at low frequencies [P. Wilson, in *Auditory Physiology and Perception*, Y. Cazals, K. Horner, L. Demany, Eds. (Pergamon, Oxford, 1992)]. In humans, the cochlear linearity has been measured indirectly with the level dependence of the auditory filter bandwidths, which showed that the cochlea is essentially linear at 125 Hz, with no level effect on the bandwidths of the auditory filter centered at this frequency. The cochlea becomes more nonlinear as frequency is increased from 250 Hz to 1000 Hz [S. Rosen and D. Stock, *J. Acoust. Soc. Am.* **92**, 773 (1992)]. This boundary of low versus high frequency is similar to that suggested by the present study.

14. For brevity, noncritical constants are intentionally neglected, and the brain is assumed to perform an exponential transformation in the model. The specific exponential mechanism could be a double-log transformation as suggested by MacKay (*3*).

15. R. V. Shannon, *Hear. Res.* **11**, 157 (1983).

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[URE3] as an Altered *URE2* Protein: Evidence for a Prion Analog in *Saccharomyces cerevisiae*

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A cytoplasmically inherited element, [URE3], allows yeast to use ureidosuccinate in the presence of ammonium ion. Chromosomal mutations in the *URE2* gene produce the same phenotype. [URE3] depends for its propagation on the *URE2* product (Ure2p), a negative regulator of enzymes of nitrogen metabolism. *Saccharomyces cerevisiae* strains cured of [URE3] with guanidium chloride were shown to return to the [URE3]-carrying state without its introduction from other cells. Overproduction of Ure2p increased the frequency with which a strain became [URE3] by 100-fold. In analogy to mammalian prions, [URE3] may be an altered form of Ure2p that is inactive for its normal function but can convert normal Ure2p to the altered form. The genetic evidence presented here suggests that protein-based inheritance, involving a protein unrelated to the mammalian prion protein, can occur in a microorganism.

Prions are infectious proteins, a concept that arose from studies of the spongiform encephalopathies, including scrapie of sheep, human kuru, and Creutzfeldt-Jakob disease (*1*). A prion protein is an altered form of a normal cellular protein that causes a detectable phenotype or disease in the affected individual. The altered (prion) protein transmits the disease to a new individual, without transmitting any genetic material, by inducing the normal cellular form of the new host to change to the prion form. As one would predict, a transgenic mouse lacking the cellular prion gene (*PrP*), and hence its protein product, is unable to propagate the prion and is resistant to its disease-inducing effects (*2*).

Yeast viruses are generally passed from cell to cell by cytoplasmic mixing such as occurs when cells mate. Such events are

sufficiently frequent in nature that known yeast viruses are found in most strains examined (*3*). A yeast prion would be expected to have the same kind of infectivity and similarly appear as a non-Mendelian genetic element, but with certain special characteristics (Fig. 1).

Aspartate transcarbamylase is an enzyme in the pyrimidine biosynthetic pathway that produces ureidosuccinate from carbamyl phosphate and aspartate (*4*). Mutants in aspartate transcarbamylase can grow if supplemented with ureidosuccinate, but its uptake is repressed by ammonium (*5*). In 1971, Lacroute, starting with a strain lacking this enzyme, isolated mutants called URE (for ureidosuccinate) that could grow on ureidosuccinate despite the presence of ammonium (*6*).

One group of recessive mutants when crossed with wild type showed the 2+:2− meiotic segregation typical of mutation in a single chromosomal gene. These mutants defined the chromosomal *URE2* gene (*6*) whose normal role is repression of nitrogen

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catabolism genes (7). Another mutant, called [URE3], was dominant and, as will be described below, appeared to be a non-chromosomal genetic element (6).

One can easily isolate [URE3] "mutants" by plating an aspartate transcarbamylase mutant on ureidosuccinate (5, 6, 8). In one experiment, [URE3] derivatives of strain 3381 (MATα kar1 ura2 leu2 trp1 L-A M$_1$ [ure3]) arose at a frequency of 10^{-5} in the absence of mutagenesis. [URE3] derivatives are stable during prolonged subcloning; in one experiment, all 24 subclones tested were still [URE3] after 60 generations of growth on nonselective (YPAD) medium. The [URE3] strains grow slightly slower than their isogenic [ure3] parents on minimal medium supplemented with uracil, and yet [URE3] is stably maintained.

Three lines of evidence indicate that [URE3] is a nonchromosomal genetic element. First, when a [URE3] strain is mated with a wild-type strain ([ure3]), an excess of [URE3] meiotic segregants is observed over the 2[URE3]:2[ure3] expected for a chromosomal locus (6, 9). I have confirmed this: In two crosses of the type [URE3] × [ure3], segregation was the classical 4[URE3]:0 pattern typical of non-Mendelian elements (Table 1). Second, [URE3] is efficiently transmitted by cytoplasmic mixing without nuclear fusion (cytoduction) (9), which I also confirmed. For example,
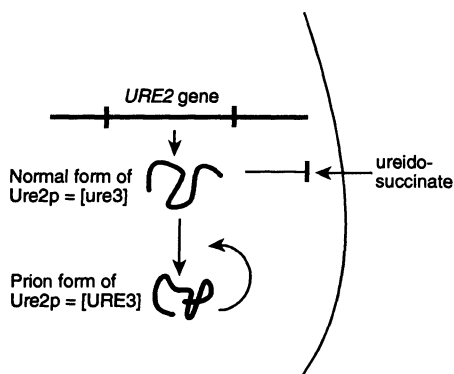

Fig. 1. Model of [URE3] as a prion form of Ure2p. The prion form of Ure2p can catalyze the conversion of the normal form of Ure2p to the prion form but cannot block ureidosuccinate uptake as does the normal form. If Ure2p is a GST as suggested by its sequence, this modification of normal Ure2p may be transfer of glutathione to form GS-Ure2p. The [URE3] state is initiated when some Ure2p molecules are (spontaneously) modified. Then those molecules modify all the Ure2p in that cell, and ureidosuccinate uptake is no longer prevented. The [URE3] state is dominant and self-propagating. The [ure3] state is established when there is no abnormal Ure2p able to modify newly synthesized normal Ure2p. This is recessive and self-maintaining. Guanidine "cures" [URE3] by inactivating the Ure2p-converting activity of the prion form.

all 18 clones of strain 3400 (initially [ure3] ρ°) that received cytoplasm from strain 3560 ([URE3]U22) were [URE3]. Third, [URE3] is said to be "cured" by growth of cells on media containing guanidine HCl (10). I found that 5 mM guanidine HCl added to YPAD plates allowed nearly normal growth of all strains but 100% curing of [URE3]. That cell growth and plating efficiency are not affected while complete curing is produced shows that this is not selection of [ure3] cells. This treatment has no effect on the ability of ure2Δ strains or the inability of a wild-type strain to use ureidosuccinate.

That the [URE3] element requires the URE2 gene for its propagation was shown by Aigle and Lacroute (9) with meiotic segregation and cytoduction experiments. This requirement is striking because the absence of the URE2 product has the same phenotype as the presence of the [URE3] element. In contrast, the absence of the chromosomal gene MIP1, a DNA polymerase required for propagation of ρ (the mitochondrial genome) (11) has, as one would expect, the opposite phenotype of the presence of ρ.

To confirm this result by a different method, I used the scheme described in Table 2. In effect, a URE2 [URE3] strain was made ure2, then returned to being URE2, and tested for [URE3] by its ability to use ureidosuccinate. The plasmid YEp351-URE2 (12) was introduced into either of two ure2Δ strains to make them unable to grow on ureidosuccinate in place of uracil. Several [URE3] derivatives were selected, purified, and found to be mitotically stable. Growth of these strains on rich

Table 1. Meiotic segregation of [URE3].

Cross	Parents	Ability (+) or inability (−) to utilize ureidosuccinate	
		Dip-loids	Segre-gation (tetrads)*
4402	3382 × 3383	−	4−:0 (12)
4403	3382 × 3383†	+	4+:0 (12)
4405	4402-2A × 4403-6C‡	+	4+:0 (12)

*In most crosses of a [URE3] strain with a wild-type strain ([ure3]), meiotic tetrads were produced in which three or four of the spore clones were [URE3] (6, 9). I have confirmed this, observing crosses in which segregation was a mixture of 2[URE3]:2[ure3], 3[URE3]:1[ure3], and 4[URE3]:0. The crosses shown here were chosen because they display the classical 4[URE3]:0 pattern typical of non-Mendelian elements. †From diploid 3382 (MATα kar1 ura2 leu2 trp1 [ure3]) × 3383 (MATa kar1 ura2 leu2 his⁻ [ure3]) a ureidosuccinate-utilizing colony was selected which was purified and sporulated. ‡Cross 4405 is a cross of a [ure3] segregant of cross 4402 with a [URE3] segregant of cross 4403.

medium allowed loss of YEp351-URE2, producing ure2 cells that could use ureidosuccinate whether they still had [URE3] or not. To test whether [URE3] had been lost or not, I reintroduced YEp351-URE2 and tested the cells. These strains could not use ureidosuccinate, which showed that [URE3] had been lost while the cells were ure2 (Table 2), confirming for the ure2Δ null allele the results of Aigle and Lacroute (9). As controls, URE2 [ure3] and URE2 [URE3] strains carrying YEp351-URE2 were treated in the same way. The results (Table 2) showed that none of the manipulations resulted in loss or gain of [URE3] in the URE2 (wild-type) strains.

Guanidine HCl efficiently cures [URE3], but when a cured, purified clone was placed under selective conditions, [URE3] colonies were again found. This was shown for

Table 2. Requirement of URE2 for propagation of [URE3]. The ure2Δ strains 3422 and 3423 (both MATaleu2 ura2 kar1 his⁻ ure2::URA3) were transformed with YEp351-URE2 to make them unable to utilize ureidosuccinate. [URE3] derivatives were then selected on SD + his + ureidosuccinate. I tested each [URE3] derivative for stability by subcloning on SD + his + uracil, and each of 20 subclones were still able to utilize ureidosuccinate. After the [URE3] clones were purified, they were subcloned on YPAD medium which allowed them to lose YEp351-URE2. These Leu⁻ clones were purified on YPAD, and YEp351-URE2 was then reintroduced, and four transformants from each original [URE3] isolate were purified and tested for their ability to utilize ureidosuccinate. As controls [URE3] and [ure3] derivatives of strain 3389 (MATa kar1 ura2 leu2 his⁻ YEp351-URE2 [ure3]) carrying the same YEp351-URE2 were treated similarly starting at step B in the scheme below.

Transform with
YEp351-URE2   A
ure2Δ [ure3] ⟶ ure2Δ [ure3] YEp351-URE2

Select   B   Allow loss of
[URE3] ⟶ ure2Δ [URE3] YEp351-URE2 YEp351-URE2 ⟶

                 C   Again transform with
                       YEp351-URE2
ure2Δ [URE3]?? ⟶

         D   Test use of
ure2Δ [URE3]?? YEp351-URE2 ⟶ ureidosuccinate

Strain	Chromo-somal URE2 geno-type	[URE3] isolate B	Ability of D to use ureido-succinate
3422	ure2Δ	[URE3]U1	−
	ure2Δ	[URE3]U2	−
	ure2Δ	[URE3]U2	−
	ure2Δ	[URE3]U4	−
3423	ure2Δ	[URE3]U2	−
	ure2Δ	[URE3]U3	−
3389	URE2	[URE3]U22	+
3389	URE2	[ure3]	−

strain 3389 [URE3]U22 and the [URE3] derivative of strain 3382 × 3383 whose meiotic segregation is described in Table 1. These new [URE3] derivatives were, like the original isolate, curable again by guanidine HCl and transferable by cytoduction.

The presence of URE2 on a high copy plasmid results in increased Ure2p and a 100-fold increase in the frequency of change to [URE3]. The URE2 gene was introduced into either of two wild-type [ure3] hosts as YEp351-URE2, a 2-μm–based high copy plasmid (Table 3) (12). Immunoblot analysis of Ure2p with antibody raised in rabbits against a glutathione-S-transferase (GST)–Ure2p fusion protein produced in Escherichia coli showed that Ure2p was overproduced at least 10-fold (13). These cells had a frequency of ure-idosuccinate-utilizing derivatives that was elevated about 100-fold as compared with the same strain carrying the vector alone (Table 3). Each of 24 such colonies were purified and efficiently transferred the ure-idosuccinate utilization trait by cytoduction to a [ure3] strain, indicating that these isolates were [URE3]. All were also found to be curable at high efficiency by growth on 5 mM guanidine HCl. These results suggest that the overproduction of Ure2p increased the frequency of generation of the [URE3] state.

The high copy plasmid carrying URE2 was eliminated from 16 of these [URE3] derivatives by growth on YPAD plates. Single colonies lacking the plasmid were tested again for [URE3]. [URE3] was found to remain in all cases. This indicates that these [URE3] elements do not need the URE2 plasmid to be maintained, that the alteration was not due to a mutant URE2 on the plasmid, and that it was the conversion from [ure3] to [URE3] that was stimulated by the high copy number of URE2, and presumably by elevation of Ure2p.

To test further whether overproduction of Ure2p induces acquisition of [URE3], I made YEp351G-URE2 (12) with the URE2 gene under control of the GAL1 promoter. This plasmid (or the vector YEp351G as control) was introduced into a [ure3] strain, and transformants were grown on galactose to induce transcription of URE2 or glucose to prevent transcription. Only cells that had been grown on galactose showed the increased conversion to [URE3], in this case by about 100-fold (Table 4). This showed that it was not simply the URE2 gene in high copy that produced the change to [URE3], but that a gene product was necessary.

To test whether Ure2p was the product producing the change to [URE3], I introduced translation termination mutations in place of codons 105 or 115 of the 354-residue URE2 gene. Neither modified gene could induce the change to [URE3] (Table 4), which indicated that the protein rather than the transcript itself was inducing the change to [URE3].

In attempting to explain the [URE3] non-Mendelian genetic element (Fig. 1), one must consider its three unusual features: reversible "curability," a requirement for Ure2p for propagation, and increased frequency of generation as a result of increased expression of Ure2p. That it can be "cured" with guanidine and then can be reisolated suggests that the "curing" was not the elimination from the cell of a nonchromosomal replicon. Rather, the normal state, [ure3], and the abnormal state,

Table 4. Overexpression of Ure2p increases the frequency of [URE3]. Into strain 3469 (3382 × 3383) was introduced either the vector YEp351G or YEp351G-URE2, in which URE2 is under control of the GAL1 promoter. In experiment 2, two nonsense mutants of URE2 on the plasmid were used as well: YEp351G-URE2oc has UAA at codon 115 and YEp351G-URE2op has UGA at codon 105. For each plasmid, four transformants were grown for 24 hours on either SD or SGal supplemented with_ uracil. Cells were then plated to select for the ability to utilize ureidosuccinate. The average of results for the four transformants with each plasmid is shown, but each transformant showed essentially the same result. Dex, dextrose; Gal, galactose.

Plasmid	Carbon source	[URE3] colonies per 10^6 cells	Fold increase
Experiment 1			
YEp351G	Dex	7	1
YEp351G	Gal	30	4
YEp351G-URE2	Dex	9	1
YEp351G-URE2	Gal	2101	233
Experiment 2			
YEp351G	Gal	7	1
YEp351G-URE2	Gal	247	35
YEp351G-URE2oc	Gal	11	1
YEp351G-URE2op	Gal	9	1

[URE3], must be alternate states. Of what are they alternate states?

What suggests itself at first is Ure2p because the phenotype of [URE3] is that of the absence of Ure2p. Drillien and Lacroute (5, 14) showed that all the aspects of the ure2⁻ phenotype that they tested were reproduced in [URE3] strains. That overproduction of Ure2p increases the frequency of the change to [URE3] and that URE2 is necessary for the propagation of [URE3] strongly support this idea as well. Overproduced Ure2p presents a larger target for the sporadic change to [URE3]. Guanidinium curing presumably works by inhibiting the Ure2p altered form's conversion of normal Ure2p to the altered form. As the cells form a colony, the altered form is diluted out and lost.

Could [URE3] be the defective interfering form of some wild-type nonchromosomal replicon that normally is necessary to block ureidosuccinate uptake and depends on URE2 for its propagation? This would explain the dominance relationships, the loss of [URE3] in ure2 mutants, and the phenotype of ure2 strains. But then introducing the URE2 gene into a ure2Δ mutant (Table 2) would not prevent it from utilizing ureidosuccinate, because the hypothetical wild-type replicon would not have been restored even though the URE2 gene was restored. Thus, this model is ruled out.

Prion proteins have been described in many vertebrates (1). These proteins are all highly homologous with each other, and with varying efficiencies, a prion protein from one species can convert the normal form of another species into a prion form.

The properties of [URE3] suggest a broader definition of a prion, to include any protein that indefinitely propagates an altered form of itself (without the continued presence of a special external stimulus) and is transmissible. This may include some self-modifying enzymes as well as proteins that promote conformational change of their normal form. Ure2p is a negative regulator of nitrogen catabolic enzyme transcription that works by inactivating the positive transcription regulator, Gln3p, which directly acts on the regulated genes (7, 15, 16). Ure2p has substantial homology to GSTs (16), and studies of the mechanism of action of Ure2p indicate that it modifies Gln3p posttranslationally rather than regulating its synthesis (15–17). This suggests that the normal form of Ure2p may glutathionate Gln3p, whereas the form found in [URE3] strains may glutathionate itself, but be unable to modify Gln3p.

My preliminary attempts to isolate Ure2p indicate that it is in a nonnuclear particulate fraction, and I have not yet been able to detect electrophoretic differences between the Ure2p isolated from

Table 3. URE2 on a high copy plasmid increases the frequency of [URE3] colonies. Each transformant colony was grown on H-leu medium and then 3.3 × 10^6 cells were plated on SD + his + ureidosuccinate to select for cells with the [URE3] element. Host strains 3383 and 3385 (both MATa kar1 ura2 leu2 his⁻ [ure3]) were used.

Colony	Host	Plasmid	[URE3] colonies per 10^6 cells
1	3383	YEp351	17
2	3383	YEp351-URE2	630
3	3383	YEp351-URE2	1793
4	3385	YEp351	10
5	3385	YEp351	20
6	3385	YEp351-URE2	1380
7	3385	YEp351-URE2	1680

[URE3] strains and that from [ure3] cells. It is also vital to show that the isolated Ure2p can transmit the [URE3] character. But such data in the scrapie field has not quelled controversy concerning the nature of the infectious entity because the low ratio of infectious units to molecules makes ruling out a nucleic acid component difficult.

If, as suggested here, prions are a more general phenomenon than the essentially single mammalian case, are there not other phenomena which could be explained in this way? [PSI] is a non-Mendelian genetic element of S. cerevisiae discovered by Cox by its enhancement of ochre suppression [reviewed in (18, 19)]. [PSI] is reversibly curable (like [URE3]). The PNM2 gene (PSI no more), necessary for propagation of [PSI], is identical to sup35/sal3/SUP2/suf12 and is intimately involved in translational fidelity, and many pnm2 mutants have a [PSI]-like phenotype (like ure2 mutants having a [URE3]-like phenotype) (18, 19). Finally, PNM2 on a high-copy plasmid results in frequent de novo generation of [PSI] (20). The logical parallels with [URE3] and URE2 make a compelling case that [PSI] is a prion form of the PNM2 protein.

REFERENCES AND NOTES

1. D. C. Gajdusek, Eur. J. Epidemiol. 7, 567 (1991); S. B. Prusiner, Science 252, 1515 (1991); C. Weissmann, Nature 352, 679 (1991); J. S. Griffith, ibid. 215, 1043 (1967); S. B. Prusiner, Science 216, 136 (1982).
2. H. Bueler et al., Cell 73, 1339 (1993); S. B. Prusiner et al., Proc. Natl. Acad. Sci. U.S.A. 90, 10608 (1993).
3. R. B. Wickner, Annu. Rev. Microbiol. 46, 347 (1992).
4. Nomenclature: For URE2, as for all chromosomal genes, the dominant allele is shown in capital italics. The wild-type allele is dominant and the mutant, ure2, is recessive. Ure2p means the protein product of URE2. The brackets in [URE3] indicate that it is a non-Mendelian element and the capitals that it is dominant, although [URE3] strains are isolated as "mutants." The wild-type (recessive) state is [ure3]. Aspartate transcarbamylase is encoded by URA2, so the ability of ura2 mutants to grow on ureidosuccinate in the presence of ammonia is used to assess URE2 and [URE3].
5. R. Drillien and F. Lacroute, J. Bacteriol. 109, 203 (1972).
6. F. Lacroute, ibid. 106, 519 (1971).
7. B. Magasanik, in The Molecular and Cellular Biology of the Yeast Saccharomyces, E. W. Jones, J. R. Pringle, J. R. Broach, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992), vol. 2, pp. 283–317.
8. Assay of [URE3] and genetic manipulations: Minimal medium (SD) and rich medium (YPAD) were as described (21). SGal is SD with 2% galactose in place of dextrose. Strains carrying the [URE3] element or a mutation in the chromosomal URE2 gene are able to take up ureidosuccinate in the presence of either ammonium ion or glutamine to supply the defect in a ura2 mutant. The assay medium routinely used was SD to which was added ureidosuccinate (100 μg/ml) and any amino acids required by the strain to be tested. This medium had ammonium as the nitrogen source. Transfer of [URE3] by cytoplasmic mixing (cytoduction) was carried out as described (22). The designation ρ° means that the strain lacks mitochondrial DNA. Strains were cured of [URE3] by streaking for single colonies on YPAD plates containing 5 mM guanidine HCl. Single colonies were tested for utilization of ureidosuccinate. Generally all were unable to do so.
9. M. Aigle and F. Lacroute, Mol. Gen. Genet. 136, 327 (1975).
10. M. Aigle, personal communication.
11. F. Foury, J. Biol. Chem. 264, 20552 (1989).
12. Plasmid construction and antibodies: YEp351-URE2 was constructed by the insertion of the 2.7-kb Sal I–Bgl II fragment of p1-XS carrying URE2 (16) into YEp351 (23) cut with Bam HI and Sal I. The same URE2 fragment inserted into Sal I–Bam HI–cut pRS315 (24) produced p532, which was mutagenized (25) with the oligonucleotide UG = 5'-GACGGAGCGCATTGGATCCAT-TCGACACTTGGTTG-3' to place a Bam HI site just 3' of the initiator AUG of URE2. The 1.2-kb Bam HI–Eco RI fragment of the resulting plasmid, p578, carrying most of URE2 was inserted into pGEX-2T (Pharmacia) cut with the same two enzymes to produce an in-frame fusion with GST (p580). This fusion protein was used to immunize rabbits (Berkeley Antibody Company). To place URE2 under galactose control, plasmid YEp351G with the 0.9-kb GAL1 promoter inserted between the Eco RI and Bam HI sites of YEp351 (26) was used. The plasmid p532 was mutagenized with the oligonucleotide UB = 5'-GTTATTCATCATT-TGGGATCCAACTTAATTTGCAGC-3' which replaces bases 173 to 178 (just 5' of the initiator AUG) with a Bam HI site, and YEp351G-URE2 was made by insertion of the 2.1-kb fragment from URE2 base 179 to the Xba I site 3' of the URE2 open reading frame into YEp351G cut with Bam HI and Xba I. The bacteriophage f1 ori from pDM1 (27) on a 0.5-kb Sal I fragment was inserted into the Sal I site just 3' of the URE2 gene in YEp351G-URE2. The resulting plasmid, p649, was mutagenized with two oligonucleotides: 495 = 5'-GGTTGTTCTTGAATTCATTTTGTAATTCTG-3', replacing amino acid codon 105 of URE2 with UGA (opal) (p652 = YEp351G-URE2op), and 525 = 5'-CCTGTGAGAGAAAGCTTAATATCCCTCCA-GT-3', which replaces codon 115 with UAA (p655 = YEp351G-URE2oc). All mutations were confirmed by sequencing.
13. R. B. Wickner, unpublished data.
14. R. Drillien, M. Aigle, F. Lacroute, Biochem. Biophys. Res. Comm. 53, 367 (1973).
15. W. E. Courchesne and B. Magasanik, J. Bacteriol. 170, 708 (1988).
16. P. W. Coschigano and B. Magasanik, Mol. Cell. Biol. 11, 822 (1991).
17. K. H. Kim and S. G. Rhee, J. Biol. Chem. 263, 833 (1988).
18. B. S. Cox, M. F. Tuite, C. S. McLaughlin, Yeast 4, 159 (1988).
19. B. S. Cox, in The Early Days of Yeast Genetics, M. N. Hall and P. Linder, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), pp. 219–239.
20. Y. O. Chernoff, I. L. Derkach, S. G. Inge-Vechtomov, Curr. Genet. 24, 268 (1993).
21. F. Sherman, in Guide to Yeast Genetics and Molecular Biology, C. Guthrie and G. R. Fink, Eds. (Academic Press, San Diego, 1991), vol. 194, pp. 3–21.
22. S. P. Ridley, S. S. Sommer, R. B. Wickner, Mol. Cell. Biol. 4, 761 (1984).
23. J. E. Hill et al., Yeast 2, 163 (1986).
24. R. S. Sikorski and P. Hieter, Genetics 122, 19 (1989).
25. T. A. Kunkel, Proc. Natl. Acad. Sci. U.S.A. 82, 488 (1985).
26. B. Benton et al., Mol. Cell. Biol. 10, 353 (1990).
27. D. A. Mead, E. S. Skorupa, B. Kemper, Nucleic Acids Res. 13, 1103 (1985).
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Requirement of Vascular Integrin αvβ3 for Angiogenesis

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Angiogenesis depends on the adhesive interactions of vascular cells. The adhesion receptor integrin $\alpha_v\beta_3$ was identified as a marker of angiogenic vascular tissue. Integrin $\alpha_v\beta_3$ was expressed on blood vessels in human wound granulation tissue but not in normal skin, and it showed a fourfold increase in expression during angiogenesis on the chick chorioallantoic membrane. In the latter assay, a monoclonal antibody to $\alpha_v\beta_3$ blocked angiogenesis induced by basic fibroblast growth factor, tumor necrosis factor–α, and human melanoma fragments but had no effect on preexisting vessels. These findings suggest that $\alpha_v\beta_3$ may be a useful therapeutic target for diseases characterized by neovascularization.

The growth of new blood vessels, or angiogenesis, plays a key role in development, wound repair, and inflammation. This process also contributes to pathological conditions such as diabetic retinopathy, rheumatoid arthritis, and cancer (1–6), and there has been much interest in developing therapeutic agents that inhibit angiogenesis in these contexts. Identification of the molecules that regulate angiogenesis is critical to the success of such targeted therapies.

Angiogenesis is characterized by the invasion, migration, and proliferation of smooth muscle and endothelial cells; thus, it seems likely that vascular cell adhesion molecules contribute to its regulation (2,

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