

Prions of Yeast and Fungi

PROTEINS AS GENETIC MATERIAL*

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The genetic properties of [URE3] and [PSI], two non-chromosomal genetic elements of *Saccharomyces cerevisiae*, indicated that they were infectious proteins (prions) (1). Subsequent studies have supported this proposal, and the genetic criteria we proposed have been used in the discovery of another new prion, [Het-s], in the filamentous fungus *Podospora anserina* (2). The prion hypothesis has long been an intriguing explanation of the transmissible spongiform encephalopathies, such as scrapie, Creutzfeldt-Jakob disease, and “mad cow disease” (3–5) (reviewed in Refs. 6 and 7). Studies using *Saccharomyces* and *Podospora* have provided evidence of a type not available from studies of scrapie that there can be such a thing as an infectious protein. This work also revealed that prions can be the basis for inherited traits and initiated the use of the powerful yeast system to study this phenomenon. Here we review the basis for the proposal that [URE3], [PSI], and [Het-s] are prions of the chromosomally encoded Ure2p, Sup35p, and Het-s protein, respectively. We also review the properties of [URE3] and [Het-s]. Further studies of [PSI] are reviewed by Liebman and Derkatch in the following minireview (8), and other reviews of these subjects have appeared (9–12).

[URE3], a Non-Mendelian Genetic Element Affecting Nitrogen Catabolism

Because ureidosuccinate (USA)¹ an intermediate in uracil biosynthesis, happens to resemble allantoate, a poor but usable nitrogen source, USA uptake is repressed by good nitrogen sources (Fig. 1) (reviewed in Refs. 13 and 14).

Lacroute and co-workers (15, 16) discovered *ure2* by selecting mutants able to take up USA on media with a good nitrogen source (ammonia). The USA uptake of one isolate was dominant, showed irregular segregation in meiosis (17), and was transmissible by cytoplasmic mixing (18), confirming its determination by a non-chromosomal gene that Lacroute named [URE3].

Three Genetic Criteria for a Prion Illustrated by [URE3]

Yeast viruses are found as nonchromosomal genetic elements (19) and do not venture out of the cell but are able to become widespread as a result of transmission through mating. We likewise expect infectious proteins of yeast to be found as

non-chromosomal genetic elements. We proposed three genetic criteria that should distinguish a prion from a nucleic acid replicon (1) (Fig. 2). A prion is an altered form of a normal chromosomally encoded protein. This prion form has lost its normal function but has acquired the ability to convert the normal form of the protein into this same abnormal (prion) form.

Reversible Curability—If a prion can be cured, it should be possible for it to arise again in the cured strain at some low frequency, because the same event that initially gave rise to the prion could occur again. [URE3] can be cured by the growth of cells on media containing 1–5 mM guanidine HCl, but from cured clones [URE3]-carrying colonies can again be isolated (1). These [URE3] clones arise at frequencies similar to the 10^{-6} frequency with which [URE3] arises in most wild-type strains.

Overproduction of the Normal Protein Increases the Frequency of Prion Formation—Because the initial prion formation event is a spontaneous change of the protein, it is expected that overproducing the normal protein will increase the number of molecules that are candidates to undergo this change. Indeed, overproduction of Ure2p increases the frequency of *de novo* generation of [URE3] by 20–200-fold (1).

Phenotype Relationship of Prion and Mutation of the Gene for the Protein—Because the prion is propagated by conversion of the normal form to the prion form, mutations of the chromosomal gene for the protein that prevent it being synthesized will prevent propagation of the prion. So the chromosomal gene for the protein will be seen (perhaps discovered) as a gene necessary for propagation of the non-Mendelian genetic element. But unlike such genes necessary for propagation of mitochondrial DNA (*pet*) or of the L-A dsRNA virus (*mak*), the phenotype of the mutant should be the *same* as the phenotype of the presence of the non-Mendelian genetic element. This is because either the presence of the prion or the mutation in the gene for the protein results in a deficiency of the normal protein.

The *ure2* mutants have the same phenotype as [URE3] strains (17), and these *ure2* mutants cannot propagate [URE3] (1, 18). Thus, [URE3] satisfies all three genetic criteria to be a prion form of Ure2p.

The genetic criteria satisfied for yeast prions and, in two of three cases, by the *Podospora* prion are not yet satisfied by the transmissible spongiform encephalopathies. Thus, these new findings have provided a major foundation for the notion that there can be such a thing as an infectious protein.

[PSI] Satisfies the Genetic Criteria as a Prion Form of Sup35p

Sup35p is a subunit of the translation release factor that recognizes termination codons and releases the completed peptide from the last tRNA. [PSI] is a non-Mendelian genetic element that, like *sup35* mutations, increases the strength of weak suppressor tRNAs (20, 21). We pointed out that [PSI], like [URE3], satisfies all three genetic criteria to be a prion form of Sup35p (1). [PSI] may be cured by various agents (22, 23) but from the cured strains may again be obtained clones in which [PSI] has arisen *de novo* (24). Overproduction of Sup35p increases the frequency with which [PSI] arises about 100-fold (25). Finally, the phenotype of *sup35* mutants is similar to that of [PSI], and Sup35p is necessary for the propagation of [PSI]

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¹ The abbreviation used is: USA, ureidosuccinate.

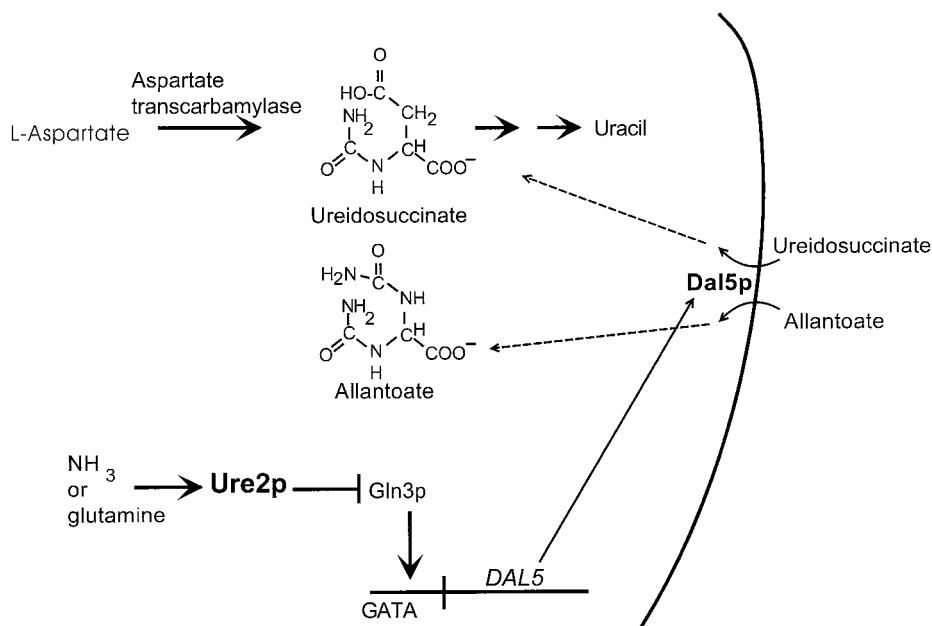


FIG. 1. Ure2p is a regulator of nitrogen catabolism that indirectly blocks uptake of ureidosuccinate, an intermediate in uracil biosynthesis. Yeast growing on a rich nitrogen source, such as ammonia or glutamine, repress transcription of enzymes and transporters needed for utilization of poor nitrogen sources. Ure2p senses the availability of a rich nitrogen source and blocks the action of Gln3p, a positive transcription regulator of many genes whose products facilitate utilization of poor nitrogen sources (37, 38). Among these genes is *DAL5*, encoding the allantoate importer (39). The incidental chemical similarity of ureidosuccinate to allantoate makes the former a substrate for the allantoate importer, Dal5p (40). USA (*N*-carbamyl aspartate) is an intermediate in uracil biosynthesis, the product of aspartate transcarbamylase. Cells blocked in this enzyme can take up ureidosuccinate to make uracil only if they are growing on a poor nitrogen source. Lacroute and co-workers (15, 16) found that chromosomal *ure2* mutants, selected for ability to take up USA on a rich nitrogen source (ammonia), were unable to repress enzymes for utilization of poor nitrogen sources.

(26, 27). Further discussion of [PSI] will be found in the mini-review by Liebman and Derkatch (8).

Further Support for the Prion Model for [URE3]

Protease Resistance of Ure2p in [URE3] Strains—If [URE3] is a prion form of Ure2p, it is expected that there will be a structural difference of some kind between Ure2p in wild-type and [URE3] strains. In fact Ure2p is more protease-resistant in extracts of [URE3] strains than wild-type strains (28). The 40-kDa Ure2p is quickly and completely degraded, but Ure2p in extracts of [URE3] cells is first partially degraded to species of 30 and 32 kDa before eventually being completely degraded.

[URE3] Is Not a Stable Regulatory Circuit—Ure2p is a transcription regulator, and stable transcription circuits are known in some cases to produce inheritable (“epigenetic”) traits (29). However, the frequency with which [URE3] arises is independent of the nitrogen-repressed or derepressed state of cells (30). Moreover, [URE3] can be propagated in cells that are either repressed or derepressed (30). Finally, as discussed below, the parts of the Ure2p molecule responsible for prion propagation and nitrogen regulation are completely distinct (28).

[URE3] Really Arises de Novo—[URE3] is isolated as an apparent mutant; it is not found in “wild-type” strains. Could [URE3] be a defective interfering derivative of a normal plasmid dependent on Ure2p for its propagation, analogous to a defective interfering virus or a “suppressive petite” deletion derivative of mitochondrial DNA? In that case, [URE3] would produce its phenotype by eliminating the normal version of the plasmid, thus explaining the dominance of [URE3]. The *ure2* mutants would also produce their phenotype by resulting in loss of the normal plasmid, and so the identity of phenotypes of [URE3] and *ure2* strains would be explained. However, replacing the *URE2* gene in a *ure2* mutant would then fail to correct its phenotypic defect, and this is not the case (31). This model would also predict that the [URE3] element could not be gen-

erated in a *ure2* strain because the parent “normal” plasmid would be already lost. In fact, introduction of a *URE2* plasmid into a *ure2* strain makes it able to become [URE3] at some low frequency (30).

This *de novo* induction of [URE3] is, in fact, because of overproduction of the Ure2 protein, not the *URE2* mRNA or the gene itself (30). Thus, [URE3] arises *de novo*, not as a derivative of a pre-existing nucleic acid replicon. Moreover, it is the Ure2 protein that leads to [URE3] arising.

Prion Domain and Nitrogen Regulation Domains of Ure2p

Deletion analysis of *URE2* showed that residues 66–354 determine the nitrogen regulation function of Ure2p (28, 31), whereas prion induction and propagation involve primarily residues 1–65 (Fig. 3) (28, 30). Although overexpression of intact Ure2p increases the frequency with which [URE3] arises by 20–200-fold (1, 28), the N-terminal 65 residues are sufficient to induce [URE3] at even higher efficiency (28). Thus, the two functions of Ure2p are attributed to separate parts of the molecule.

Deletions of parts of the C-terminal nitrogen regulation domain increase by 100-fold or more the frequency with which it converts to the prion form (28), suggesting that the C-terminal domain normally stabilizes the N-terminal domain and prevents it from converting to the prion form. Although they interact, the prion domain and nitrogen regulation domain can function completely separately in the cell when produced as separate molecules. The prion domain alone can propagate [URE3] and is not affected by the concurrent presence of separate molecules of the nitrogen regulation domain (30). Likewise, only when the nitrogen regulation domain has a covalently attached prion domain is the nitrogen regulation function affected by the presence of the [URE3] prion (30).

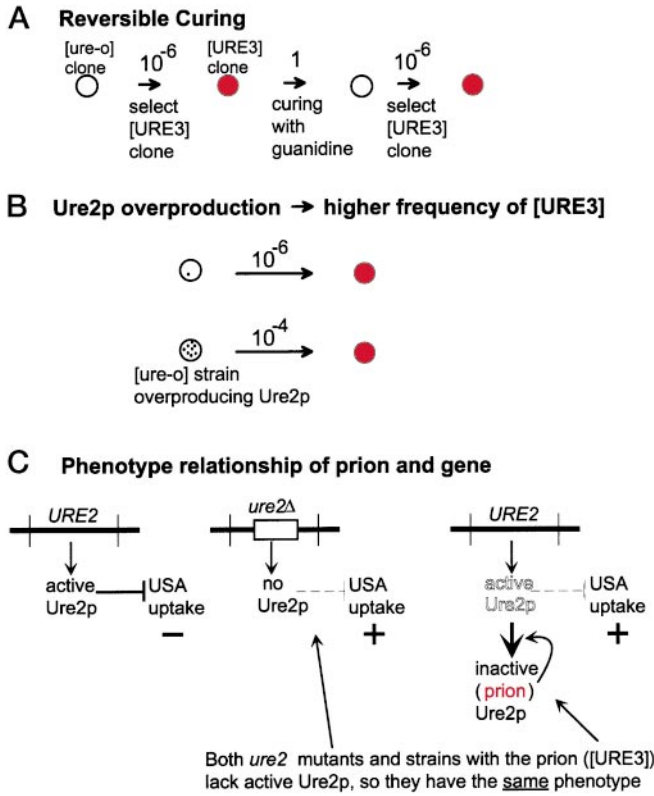
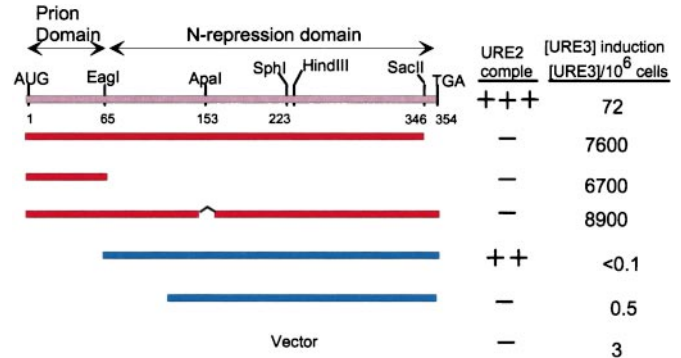


FIG. 2. Genetic evidence for prions. Similar results have been obtained for the non-Mendelian genetic element [URE3] as a prion of Ure2p and [PSI] as a prion of Sup35p. A, reversible curability. Cells cured of the element can, at some low frequency, again spontaneously acquire it. B, overproduction of Ure2p increases the frequency with which [URE3] (the prion form) arises. C, the phenotype of *ure2* mutants is the same as the phenotype of the presence of [URE3], because both lack the normal Ure2p. Moreover, *URE2* is necessary for the propagation of [URE3].

[Het-s], a Prion of *P. anserina*, Carries Out a Normal Cellular Function

A fungal colony is a syncytium so that cells can exchange cytoplasm and even nuclei. When two colonies grow together, the cellular processes (hyphae) of one colony generally fuse (anastomose) with those of the other, so the colonies can share nutrients (32). This “hyphal anastomosis” has the disadvantage that viruses present in one colony are readily transmitted throughout the other. To limit this problem, the process is completed only by closely related colonies, likely to already carry the same viruses. Colonies of *Podospora* must be identical at 8 “*het*” loci to fuse. Colonies differing in alleles at one or more *het* locus will initially fuse hyphae, but the fused hyphae undergo a rapid degeneration process with formation of a barrier to further hyphal fusions. This is called “heterokaryon incompatibility” (Fig. 4).

Recently, Coustou *et al.* (2) showed that the product of one of these loci, *het-s*, only functions when it is in a prion form. As with [URE3] and [PSI], the *Podospora* prion was first detected as a non-Mendelian (non-chromosomal) genetic element. The *het-s* locus can have either of two alleles, called *het-S* and *het-s*. Whereas *het-s* cells fuse readily with other *het-s* cells and *het-S* cells fuse with *het-S* partners, a meeting of *het-s* and *het-S* cells results in the heterokaryon incompatibility reaction. Rizet (33) found that cells with genotype *het-s* could have either of two phenotypes. One, called [Het-s], shows this heterokaryon incompatibility when meeting a *het-S* strain. The other, called [Het-s*], is compatible with both *het-s* and *het-S* partners. [Het-s] acts as a non-Mendelian genetic element and [Het-s*]



Ure2p Prion Domain:

1 MMNNNGNQVSNLSNALRQVNIIGNRNSNTTT 30
31 DQSNINFEFSTGVNNSNNSSNNNNVQNNNSGR 65

FIG. 3. Domains of Ure2p can function independently but also interact (28, 30). The N-terminal 65 residues (the “prion domain”), when overproduced, induce the *de novo* appearance of [URE3] in a strain with a normal *URE2* gene expressed at normal levels. The prion domain is necessary and sufficient to propagate [URE3] and for a molecule to be affected by [URE3]. The C-terminal nitrogen regulation domain can regulate nitrogen catabolism without the N-terminal prion domain (shown as *URE2 comple*) but requires the covalently attached prion domain to be inactivated by [URE3]. Mutations in the nitrogen regulation domain result in dramatic increases of prion-forming activity of Ure2p, suggesting that the normal C-terminal domain stabilizes the prion domain, preventing it from undergoing the prion change.

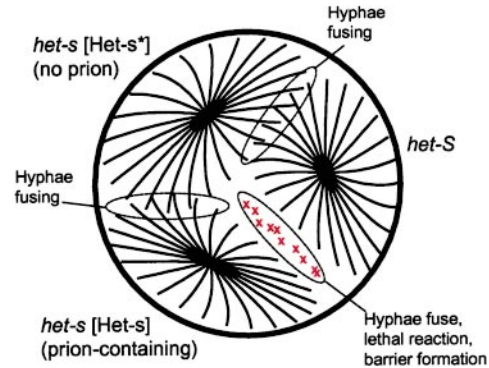


FIG. 4. The [Het-s] prion is necessary for heterokaryon incompatibility (2). Identity of alleles at the *het-s* locus is required for the cellular processes (hyphae) of different *Podospora* colonies to fuse. However, an encounter of *het-s* and *het-S* colonies will only result in the lethal reaction that comprises the incompatibility reaction if the Het-s protein is in its prion form (called [Het-s]).

as its absence (33, 34).

Coustou *et al.* (2) used two of the genetic criteria and the protease resistance of the Het-s protein in [Het-s] strains as evidence that [Het-s] is a prion form of the Het-s protein. [Het-s] can be efficiently eliminated but will spontaneously return at low frequency. Moreover, overproduction of the Het-s protein results in an increase in the frequency with which [Het-s] arises *de novo*. The *het-s* gene is necessary for the propagation of [Het-s], as expected if [Het-s] is a prion form of its product.

These findings are important in showing that a prion need not be a cause of disease but rather may be the mediator of a normal cellular function. Heterokaryon incompatibility is a normal event in the life of a fungus, with a physiological purpose.

Cortical Inheritance in Ciliates and the Parallel with Prions

Sonneborn showed that the pattern of cilia on the surface (cortex) of *Paramecium* could be altered by microsurgery or

accidents occurring during mating, events unlikely to alter the genome. These changes were transmitted to the offspring through mitosis and meiosis, in a kind of Lamarckian process (35). The implication of these studies is that the pattern of cilia on the cell surface acts as a template in the formation of the new cell. Similar phenomena have been demonstrated in other ciliates (36). This phenomenon has some formal similarity to the template function of prions believed to be the basis of prion propagation (36). Are other aspects of cell morphology heritable? Do other cellular structures act as templates in the generation of their offspring?

Comparison of Prion Systems

Whereas the prion domains of Ure2p and Sup35p are rich in asparagine and glutamine, there are no such regions in either PrP or the Het-s protein. This suggests that there will be differences in the detailed mechanisms of prion propagation, although there may yet be important similarities. Whereas [URE3] and [PSI] produce their phenotypes by inactivation of Ure2p and Sup35p, respectively, scrapie and [Het-s] are detectable because of positive activities of the altered forms of PrP and the Het-s protein, respectively.

Prospects for Future Work

The purification of Ure2p² will allow detailed structural studies of differences between the normal and prion forms of the molecule. Further dissection of prion domains and nitrogen regulation functions of Ure2p using molecular genetics are also under way.³

How widespread is the prion phenomenon? The *Saccharomyces* and *Podospora* systems should be adaptable to general screens for prion domains of any organism, and we have begun such an approach.⁴ Transmissible spongiform encephalopathies, [URE3] and [PSI], are diseases, but [Het-s] mediates a normal cellular function. We can anticipate that some other cellular functions (perhaps some aspects of differentiation and development) will be mediated by prions. The yeast prions determine inherited traits; could some inherited traits of mammals be determined by prions?

REFERENCES

1. Wickner, R. B. (1994) *Science* **264**, 566–569
2. Coustou, V., Deleu, C., Saupe, S., and Begueret, J. (1997) *Proc. Natl. Acad. Sci.*

- U. S. A.* **94**, 9773–9778
3. Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C. (1967) *Nature* **214**, 764–766
4. Griffith, J. S. (1967) *Nature* **215**, 1043–1044
5. Prusiner, S. B. (1982) *Science* **216**, 136–144
6. Weissmann, C. (1999) *J. Biol. Chem.* **274**, 3–6
7. Prusiner, S. B. (1996) in *Fields Virology* (Fields, B. N., Knipe, D. M., and Howley, P. M., eds) Vol. 2, 3rd Ed., pp. 2901–2950, Raven Publishers, Philadelphia
8. Liebman, S. W., and Derkatch, I. L. (1999) *J. Biol. Chem.* **274**, 1181–1184
9. Wickner, R. B. (1996) *Annu. Rev. Genet.* **30**, 109–135
10. Wickner, R. B., and Masison, D. C. (1996) *Curr. Top. Microbiol. Immunol.* **207**, 147–160
11. Lindquist, S. (1997) *Cell* **89**, 495–498
12. Kushnirov, V. V., and Ter-Avanesyan, M. D. (1998) *Cell* **94**, 13–16
13. Cooper, T. G. (1982) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression* (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) Vol. 2, pp. 39–99, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Magasanik, B. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces* (Jones, E. W., Pringle, J. R., and Broach, J. R., eds) Vol. 2, 2nd Ed., pp. 283–317, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Schoum, J., and Lacroute, F. (1969) *C. R. Acad. Sci.* **269**, 1412–1414
16. Drillien, R., Aigle, M., and Lacroute, F. (1973) *Biochem. Biophys. Res. Commun.* **53**, 367–372
17. Lacroute, F. (1971) *J. Bacteriol.* **106**, 519–522
18. Aigle, M., and Lacroute, F. (1975) *Mol. Gen. Genet.* **136**, 327–335
19. Wickner, R. B. (1996) *Microbiol. Rev.* **60**, 250–265
20. Cox, B. S. (1965) *Heredity* **20**, 505–521
21. Cox, B. S. (1993) in *The Early Days of Yeast Genetics* (Hall, M. N., and Linder, P., eds) pp. 219–239, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Singh, A. C., Helms, C., and Sherman, F. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 1952–1956
23. Tuite, M. F., Mundy, C. R., and Cox, B. S. (1981) *Genetics* **98**, 691–711
24. Lund, P. M., and Cox, B. S. (1981) *Genet. Res.* **37**, 173–182
25. Chernoff, Y. O., Derkach, I. L., and Inge-Vechtomov, S. G. (1993) *Curr. Genet.* **24**, 268–270
26. Ter-Avanesyan, A., Dagkesamanskaya, A. R., Kushnirov, V. V., and Smirnov, V. N. (1994) *Genetics* **137**, 671–676
27. Doel, S. M., McCready, S. J., Nierras, C. R., and Cox, B. S. (1994) *Genetics* **137**, 659–670
28. Masison, D. C., and Wickner, R. B. (1995) *Science* **270**, 93–95
29. Novick, A., and Weiner, M. (1957) *Proc. Natl. Acad. Sci. U. S. A.* **43**, 553–566
30. Masison, D. C., Maddelein, M.-L., and Wickner, R. B. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 12503–12508
31. Coschigano, P. W., and Magasanik, B. (1991) *Mol. Cell. Biol.* **11**, 822–832
32. Begueret, J., Turq, B., and Clave, C. (1994) *Trends Genet.* **10**, 441–446
33. Rizet, G. (1952) *Rev. Cytol. Biol. Veg.* **13**, 51–92
34. Beisson-Schecroun, J. (1962) *Ann. Genet.* **4**, 3–50
35. Beisson, J., and Sonneborn, T. M. (1965) *Proc. Natl. Acad. Sci. U. S. A.* **53**, 275–282
36. Grimes, G. W., and Auffderheide, K. J. (1991) *Cellular Aspects of Pattern Formation: The Problem of Assembly*, Karger, Basel
37. Mitchell, A. P., and Magasanik, B. (1984) *Mol. Cell. Biol.* **4**, 2758–2766
38. Courchesne, W. E., and Magasanik, B. (1988) *J. Bacteriol.* **170**, 708–713
39. Rai, R., Genbauffe, F., Lea, H. Z., and Cooper, T. G. (1987) *J. Bacteriol.* **169**, 3521–3524
40. Turoscy, V., and Cooper, T. G. (1987) *J. Bacteriol.* **169**, 2598–2600

² K. Taylor and R. B. Wickner, unpublished results.

³ M.-L. Maddelein and R. B. Wickner, unpublished results.

⁴ R. B. Wickner, unpublished results.