Published in final edited form as:

Mol Cell. 2020 January 16; 77(2): 266–278.e6. doi:10.1016/j.molcel.2019.10.027.

Widespread prion-based control of growth and differentiation strategies in *Saccharomyces cerevisiae*

Alan K. Itakura^{1,2,4}, Anupam K. Chakravarty^{2,4}, Christopher M. Jakobson², Daniel F. Jarosz^{2,3,5}

¹Department of Biology, Stanford University, 269 Campus Drive, Stanford, CA 94305

²Department of Chemical and Systems Biology, Stanford University, 269 Campus Drive, Stanford, CA 94305

³Department of Developmental Biology, Stanford University, 269 Campus Drive, Stanford, CA 94305

SUMMARY

Theory and experiment suggest that organisms would benefit from pre-adaptation to future stressors based on reproducible environmental fluctuations experienced by their ancestors. Yet mechanisms driving pre-adaptation remain enigmatic. We report that the [*SMAUG*⁺] prion allows yeast to anticipate nutrient repletion after periods of starvation, providing a strong selective advantage. By transforming the landscape of post-transcriptional gene expression, [*SMAUG*⁺] regulates the decision between two broad growth and survival strategies: mitotic proliferation or meiotic differentiation into a stress-resistant state. [*SMAUG*⁺] is common in laboratory yeast strains, where standard propagation practice produces regular cycles of nutrient scarcity followed by repletion. Distinct [*SMAUG*⁺] variants are also widespread in wild yeast isolates from multiple niches, establishing that prion polymorphs can be utilized in natural populations. Our data provide a striking example of how protein-based epigenetic switches, hidden in plain sight, can establish a transgenerational memory that integrates adaptive prediction into developmental decisions.

Graphical Abstract

⁵Lead contact, contact at jarosz@stanford.edu.

Author Contributions

A.I., A.K.C. and D.F.J. conceived and designed experiments; A.I. and C.M.J. performed modeling; A.I. and A.K.C. performed experiments and analyzed data; A.I., A.K.C. and senior author D.F.J. wrote the paper.

⁴These authors contributed equally.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Declarations of Interests

The authors declare no competing interests.

INTRODUCTION

Living systems endure constantly changing environments (Bell and Collins, 2008). To cope with this variability, organisms sense fluctuations in their surroundings and respond by altering their growth and survival strategies. In nature, however, environmental fluctuations are often correlated, occurring in a predictable sequence. In theory, the capacity to anticipate and pre-adapt to an upcoming stress would offer significant advantages (Tagkopoulos et al., 2008). Laboratory evolution experiments in which precise sequences of environmental stresses recur over hundreds of cycles can generate subpopulations of cells that ‘pre-adapt’ to future stressors. Continued propagation in static conditions leads to the loss of adaptive prediction, suggesting that inappropriate pre-adaptation incurs a fitness cost (Mitchell et al., 2009). The degree to which this phenomenon occurs in nature, and the underlying mechanisms involved, remain largely unexplored.

In nutrient-rich environments, the budding yeast *Saccharomyces cerevisiae* replicates rapidly via mitotic division. In this organism and many others, stress (e.g. nutrient starvation) triggers a developmental program, meiosis, that re-assorts and diversifies genetic material (Marston and Amon, 2004; McDonald et al., 2016; Miller et al., 2013; Neiman, 2011). This culminates with the formation of haploid spores, which are resistant to many stresses (Calahan et al., 2011; Coluccio et al., 2008; Smits et al., 2001). If a stressful condition is long-lasting, commitment to this program provides a strong advantage. If the stress proves transient, it would be preferable to delay meiotic commitment, and quickly resume mitotic growth after the stress ceases. Hardwiring of either strategy (via genetic mutation) allows progeny to benefit from the experience of their ancestors, but could prove maladaptive if future environmental fluctuations differ. By contrast, the ability to reversibly switch between developmental strategies could allow a population to thrive in a dynamic environment, expanding its niche. In theory, a mechanism that permits both strategies — a mode of inheritance that is simultaneously heritable and reversible — would provide significant adaptive value (Jablonka et al., 1995; King and Masel, 2007; Suzuki et al., 2012).

The unusual folding landscapes of prion proteins provide one such mechanism. Prions are stable, heritable, self-templating protein conformations that can serve as a robust mechanism for transgenerational epigenetics. In contrast to most other epigenetic states, prions are meiotically-stable, allowing for true transgenerational memory (Harvey et al., 2018). They are a reversible and lost spontaneously more frequently ($\sim 10^{-5}$ per cell division; reviewed in:

Halfmann and Lindquist, 2010; Harvey et al., 2018; Rando and Verstrepen, 2007) than mutations in DNA ($\sim 10^{-8}$ per cell division; Lang and Murray, 2008). Although their adaptive value has been controversial (Wickner et al., 2011), prions can be beneficial in many stresses (Harvey et al., 2018; Jarosz et al., 2014a; Jarosz et al., 2014b), leading to the proposal that prions could provide adaptive value as bet-hedging devices in fluctuating environments (Halfmann et al., 2010; King and Masel, 2007; Lancaster et al., 2010). Modeling suggests that this property would have been sufficient to drive evolutionary retention of several prions (Jarosz et al., 2014a; Jarosz et al., 2014b; King and Masel, 2007; Simons, 2009). Regulated, but non-infectious, protein aggregation is also emerging as an important contributor to cell physiology in processes including metabolism (Saad et al., 2017), aging (Saarikangas and Barral, 2015; Schlissel et al., 2017), pheromone response (Caudron and Barral, 2013), translation (Franzmann et al., 2018), and meiosis (Berchowitz et al., 2015).

Meiosis is one of the most complex decision-making processes in which *S. cerevisiae* engages, its efficiency varies substantially across this species (Mortimer, 2000). Common laboratory strains are notoriously poor sporulators (Gerke et al., 2006), whereas some wild strains sporulate far more readily (Liti et al., 2009; Warringer et al., 2011). Some genetic determinants of this variability have been characterized (Ben-Ari et al., 2006; Gerke et al., 2009). But what epigenetic factors contribute, if any, remain unknown.

Here we investigate the advantages of a heritable epigenetic element that promotes mitotic proliferation and delays meiosis in *S. cerevisiae*. We report that [*SMAUG*⁺], a prion formed by the *S. cerevisiae* homolog of the RNA-binding protein Smaug (encoded by the *VTS1* gene), controls this cellular decision. Modeling and experiment suggest that [*SMAUG*⁺] has adaptive value in fluctuating environments as an adaptive prediction strategy. [*SMAUG*⁺] is ubiquitous in laboratory yeast strains, which have been exposed to repeated cycles of starvation followed by nutrient repletion, where it re-shapes the post-transcriptional landscape underlying meiotic commitment. Distinct [*SMAUG*⁺] variants are widespread in wild yeast from diverse ecological niches, establishing that structural polymorphism within this prion is common in nature. Our observations provide a striking example of protein-based epigenetic inheritance hidden in plain sight, profoundly altering growth and differentiation strategies.

RESULTS

Modeling tradeoffs between mitotic and meiotic proliferation strategies

We began by modeling tradeoffs between mitotic proliferation and meiotic differentiation. In nutrient-rich conditions, *S. cerevisiae* proliferates rapidly via mitotic cell division. However, when nutrients are scarce, this diploid organism commits to sporulation, a developmental program that encompasses meiosis (Chu et al., 1998; Marston and Amon, 2004; Miller et al., 2013). In addition to genetic diversification, meiosis creates a differentiated stress-resistant cell state, a haploid spore (Calahan et al., 2011). If conditions remain stressful, facile commitment to sporulation would provide a strong survival advantage, but it could inflict a strong disadvantage if a stress is transient.

We simulated these scenarios *in silico* (Figure 1A), considering two populations of cells, ‘proliferators’ that commit to meiosis late and ‘sporulators’ that commit early. We assigned a growth advantage (μ) to efficient proliferation in nutrient-rich conditions and imposed a penalty ($1-z$) on individuals that did not rapidly sporulate in the stressful environment (Figure 1A). We assumed that both populations could achieve the same maximum sporulation efficiency (see Method Details).

We iteratively simulated the fate of mixed populations of proliferators and sporulators through multiple cycles of feast and famine, varying intrinsic (e.g., growth and death rates) and extrinsic (e.g. duration of stress) parameters (Figure 1A). Over a wide range, centring on values that have been experimentally observed in *S. cerevisiae* (see SI for further discussion).

These simulations revealed clear ‘niches’ in which it was more beneficial to be either a proliferator (i.e., $\log_{10}(P/S) > 0$) or a sporulator (i.e., $\log_{10}(P/S) < 0$) (Figure 1B, 1C and S1A). For example, we regularly observed non-monotonic behavior when varying the period of nutrient starvation. When times of scarcity were brief, proliferators flourished. Conversely, as times of scarcity lengthened, sporulators benefited. However, this advantage diminished and ultimately disappeared with lengthened periods of scarcity. The general patterns we observed were robust to changes in the initial ratio of proliferators to sporulators (Figure S1B). Critically, slight perturbations to the duration of environmental stress, as little as an hour, could switch which strategy would be more successful (Figure 1C).

The capacity to reversibly switch between strategies could then allow an organism to thrive in a wider range of environmental conditions (Lachmann and Jablonka, 1996; Lachmann and Jablonka, 1996; Lancaster et al., 2010; Lancaster and Masel, 2009). Moreover, utilizing a metastable memory of previous switching events to inform future decisions would be advantageous when environmental fluctuations exhibit long-term temporal correlations (Kussell and Leibler, 2005). Together with these previous studies, our modeling suggests that an epigenetic switch enabling oscillation between a proliferator and sporulator identity that is heritable, but also reversible, would expand the niches in which *S. cerevisiae* could thrive.

A prion-based proliferative growth strategy in laboratory yeast strains

Protein-based forms of epigenetic inheritance, e.g. prions, are an example of a heritable phenotypic switch that would allow cells to benefit from the memory of their ancestors but also be reversed more readily than a mutation. We recently showed that prions are pervasive in nature and can influence growth in a wide variety of environments (Chakrabortee et al., 2016; Halfmann et al., 2012). Repeated cycles of starvation followed by nutrient repletion are inherent to husbandry of microbial cultures. Therefore, we hypothesized that laboratory cultivation might select for a reversible epigenetic factor of the type we had modeled above.

We investigated whether the *S. cerevisiae* laboratory strain BY4741, which is widely used in systematic genetic and cell biological studies of this organism (Brachmann et al., 1998; Costanzo et al., 2016; Giaever et al., 2002; Huh et al., 2003; Winzeler et al., 1999) harbors a prion that confers a mitotic growth advantage. To eliminate prion-like elements from this

strain, we took advantage of their strong dependence on protein chaperone activity (Chernoff et al., 1995; Eaglestone et al., 2000; Masison et al., 2009; Shorter and Lindquist, 2004). Propagation of most prions requires robust Hsp70 activity. We therefore transformed derivatives of BY4741 with a plasmid-based dominant-negative allele of Hsp70 (Ssa1-K69M; Chakrabortee et al., 2016; Garcia et al., 2016; Lagaudriere-Gesbert et al., 2002), propagated these transformants for 75 generations, eliminated the plasmid, and then propagated them for an additional 25 generations to restore chaperone function. This regimen eliminates most amyloid and non-amyloid prions that have been tested (Chakrabortee et al., 2016). We hereafter refer to strains subjected to this regimen as ‘cured’.

We next examined how curing affected fitness, competing fluorescently-marked cured and uncured strains in co-culture and diluting into fresh medium every 48 hours. At each dilution, we determined the number of cured and uncured cells by flow cytometry. Cured BY4741 was reproducibly outcompeted by its uncured parent (Figure S2A). We also cured BY4743 (the diploid derivative of BY4741) and performed analogous competitions, observing an even stronger selection coefficient relative to its cured derivative ($s = 0.9\%$; Figure 2A). As a frame of reference, these values are larger than the average fitness ascribed non-essential genes (Breslow et al., 2008), and natural genetic variants in this organism (Jakobson and Jarosz, 2019; Sharon et al., 2018).

Our model predicts that a proliferative strategy would be more beneficial when periods of nutrient scarcity are shorter. To test this, we performed another competition, instead diluting the cultures every 24 hours. This resulted in a much stronger benefit for uncured cells ($s = 1.8\%$, twofold stronger than with 48 hour dilutions; Figure 2A). Thus, even modest changes in the period of environmental fluctuations can have a profound impact on the selective advantage of this chaperone dependent epigenetic element.

We next asked whether chaperone curing would also increase meiotic differentiation into a stress resistant spore, as might be predicted by our model. Cured BY4743 sporulated 3.1-fold more efficiently after 5 days than its isogenic, uncured parent (Figure 2B). Over dozens of independent propagations, we never observed spontaneous reversion of this phenotype, establishing its robust heritability. We observed a similar, curable, repressed sporulation phenotype in isolates of this strain obtained from multiple laboratories and genetic stock centers and in other widely used laboratory strains such as W303 (Figure S3A), establishing that this behavior is widespread.

To determine whether this curable epigenetic element was prion-like, we tested a defining feature of this form of inheritance: cytoplasmic transmission. We mated BY4741 (donor) to a cured, petite karyogamy deficient (*kar1 -15 rho⁻*) recipient strain. This yielded heterokaryons that received cytoplasm, but no nuclear material, from BY4741 (Figure 2C). We obtained cytoductants by selecting for haploid buds with restored mitochondrial function and a nuclear-encoded auxotrophic marker unique to the recipient strain.

We next used these recipients as donors for a ‘reverse’ cytoduction (see Method Details) to obtain karyogamy competent cells with a mixed cytoplasm, mated these ‘reverse’ cytoductants to a cured laboratory strain of the opposite mating type, and examined the

sporulation of the resulting diploids. All diploids that received the cytoplasm of uncured BY4741 sporulated poorly. Those that received the cytoplasm from cured BY4741 sporulated well (Figure 2C), establishing that the phenotype is transmitted cytoplasmically.

Finally, we examined the transmission of this trait in genetic crosses. Because they are based on heritable changes in protein conformation rather than DNA sequence, prion-based traits defy Mendelian patterns of inheritance and are passed to all meiotic progeny; in contrast, mutations are inherited by only half of the progeny (Harvey et al., 2018; Itakura et al., 2018; Lindquist et al., 1995; Wickner, 1994). We sporulated BY4743, dissected complete tetrads, and crossed all four haploid progeny to cured strains of the opposite mating type. Each of these diploid strains sporulated poorly (Figure 2D). That is, the low-sporulation phenotype showed a non-Mendelian pattern of inheritance. All four haploid progeny also exhibited a growth advantage in low glucose (Figure S2B). Based on the strong chaperone-dependence and the cytoplasmic, non-Mendelian patterns of inheritance of these phenotypes, we conclude that laboratory strains of *S. cerevisiae* harbor one or more prions that restrict sporulation and promote a proliferative growth strategy.

BY4743 harbors [*SMAUG*⁺]

In the accompanying manuscript (Chakravarty et al., submitted), we characterized [*SMAUG*⁺] as a prion that confers a proliferative advantage akin to the state we simulated above. We wondered whether a variant of [*SMAUG*⁺] exists in BY4743, promoting proliferation and restriction sporulation. Indeed, prions can exist as multiple stable variants with differing phenotypic strengths (Jarosz et al., 2014b; Tanaka et al., 2006; Toyama et al., 2007), a point to which we return to below.

To investigate, we took advantage of the fact that prion propagation requires continuous expression of the causal protein, employing a genetic strategy to transiently eliminate Vts1 expression. First, we crossed BY4741 to *vts1*⁻ cells and sporulated the ensuing heterozygous diploids. These diploids sporulated poorly (Figure S3B), consistent with the [*SMAUG*⁺] phenotype. We next dissected tetrads to obtain meiotic progeny, half wild-type and half *vts1*⁻. We mated these cells to the naive haploids that we had previously ‘cured’ of prions by transient Hsp70 inhibition (Figure 2B) and examined the sporulation of the resulting diploids. In diploids with the wild-type *VTS1* allele, where Vts1 had been constantly expressed, we observed low sporulation. By contrast, diploids derived from spores with the *vts1*⁻ deletion sporulated 5.8-fold more robustly (Figure 3A). Thus, the prion-like sporulation phenotype that is ubiquitous in laboratory yeast strains depends upon continuous expression of the Vts1 protein.

We extended this genetic crossing strategy restore Vts1 expression, sporulating and dissecting spores of these cured *WT/vts1*⁻ diploids (Figure 3B) and isolating haploid progeny that contained the wild-type *VTS1* allele. We then mated these cells to an isogenic cured strain, creating diploids isogenic to BY4743, but where one parent was cured of all prions by transient Hsp70 inhibition and the other parent was *selectively* cured of [*SMAUG*⁺] (but no other prion) by transient loss of Vts1 (denoted as [*smaug*⁻]). This selectively cured strain sporulated as efficiently as strains cured by transient chaperone inhibition (Figure 3B). Selective curing also eliminated any proliferative advantage that we observed in

both haploid and diploid strains (Figure 3C). Thus, the difference in mitotic proliferation and sporulation between BY4743 and cured BY4743 is dependent on a product of the *VTS1* gene.

Next, to establish that the low-sporulation phenotype is driven by the self-assembly of Vts1, we performed a protein transformation. We treated [*smaug*⁻] haploids with Zymolyase to generate spheroplasts that we transformed with condensates made from purified Vts1 protein (Chakravarty et al., submitted). After passaging successful transformants for ~125 generations (to eliminate by dilution any protein introduced during the transformation), we mated these strains to a cured partners and scored sporulation efficiency of the resulting diploids. Diploids derived from the Vts1 transformants sporulated poorly compared to control BSA transformants (Figure 3D). Vts1 transformants also exhibited a growth advantage in low glucose relative to controls transformed with BSA (Figure 3D). We conclude that widely used laboratory strains of *S. cerevisiae* harbor natural [*SMAUG*⁺] variants that reduce sporulation and favor a proliferative growth strategy.

Finally, we examined sporulation in the [*SMAUG*⁺] strain generated by transient exogenous overexpression (hereafter ‘induced’ [*SMAUG*⁺]; Chakrabortee et al., 2016). Induced [*SMAUG*⁺] diploids formed 4.9-fold fewer tetrads than BY4743 (Figure S3D), suggesting that it likely represents a stronger prion variant. Notably, increased *VTS1* expression (from a *GPD* promoter) also reduced sporulation in cured BY4743 cells (Figure S3C), raising the possibility that natural [*SMAUG*⁺] might activate Vts1 function.

Natural [*SMAUG*⁺] activates RNA decay function

Vts1 binds to specific hairpin RNA loops known as Smaug recognition elements (SREs) and targets transcripts containing them for degradation (Aviv et al., 2006; She et al., 2017). In the accompanying paper (Chakravarty et al., submitted), we report that Vts1 can exist in a self-templating prion conformation that accelerates target degradation. To determine how the natural prion variant impacts Vts1 activity, we turned to a well-established reporter expressing GFP with three SREs in its 3'-untranslated region (*GFP-SRE*; Aviv et al., 2006). When expressed from a galactose-inducible promoter, we observed a higher level of GFP fluorescence in the cured and selectively cured BY4741 strains than in uncured strains (Figure 4A and 4B). We observed no difference among these strains when using a near-identical reporter in which the SRE elements were permuted to abolish Vts1 binding (Aviv et al., 2003; Figure 4A and 4B). Together, these data establish that the natural [*SMAUG*⁺] prion is also a hyperactive state of the protein.

The endogenous [*SMAUG*⁺] gene expression program

The choice of mitotic or meiotic cell division is highly regulated in *S. cerevisiae* (Figure 5A). Because Vts1 is a post-transcriptional regulator of gene expression, we investigated the impact of [*SMAUG*⁺] on the meiotic transcriptome by performing mRNA-seq on natural [*SMAUG*⁺] cells and isogenic [*smaug*⁻] cells before and 14 hours after induction of sporulation. At the 14h timepoint no mature spores were yet visible in either culture, and mRNA abundance measurements from biological replicates clustered closely in principal

component analysis (Figure S4A), establishing the robustness and reproducibility of the data.

We benchmarked these data against previous studies of yeast meiosis (e.g. Chu et al., 1998). In both [*smaug*⁻] and [*SMAUG*⁺] cells, a large number of transcripts increased more than four-fold (Figure S4B). Many overlapped (279 messages, *p*-value = 1.25×10^{-298} by hypergeometric test, Table S1), especially those expressed early in meiosis (42 early transcripts vs. 17 late transcripts, *p*-value = 0.0032, Fisher's exact test; Table S1). Early transcripts were also more inducible in [*smaug*⁻] cells than in [*SMAUG*⁺] cells (Table S1, *p*-value = 0.0004, bootstrapped t-test).

We next examined quantitative differences in induction. Most differentially expressed messages were induced to a similar degree in both samples (Figure 5B). However, transcripts with annotated meiotic functions (*Saccharomyces* Genome Database [SGD]; yeastgenome.org; Cherry et al., 2012) were induced to a greater extent in the [*smaug*⁻] cells than in [*SMAUG*⁺] cells (Figure 5B). The affected transcripts included the master regulator of early steps of meiosis, *IME1*, as well as several other key players in early meiosis (e.g. *SAE3*, *DMC1* and *HED1*; Table S1; Primig et al., 2000). Thus, [*SMAUG*⁺] exerts a strong repressive effect on the post-transcriptional landscape of meiosis.

Enhanced degradation of a specific transcript drives phenotype

We investigated if [*SMAUG*⁺]'s impact on meiosis was a consequence of altered regulation of its targets, evaluating the expression of 195 transcripts annotated in SGD as having a function in meiosis (yeastgenome.org; Cherry et al., 2012). Seventy-six transcripts were significantly altered in [*smaug*⁻] cells, and a handful of these were differentially regulated in [*SMAUG*⁺] cells (Figure 5C, Table S2). The most differentially regulated, *MUM2*, was ~8-fold less abundant in [*SMAUG*⁺] cells than in [*smaug*⁻] cells.

MUM2 stood out for several more reasons. First, it is robustly upregulated in *vts1*⁻ cells (Aviv et al., 2006; She et al., 2017). Second, it harbors an SRE element that directly binds to Vts1 (Figure 5D; She et al., 2017). Third, *mum2*⁻ cells do not readily sporulate, giving rise to the gene's name: muddled in meiosis (Agarwala et al., 2012). Mum2 is a conserved subunit of the MIS (Mum2-Ime4-Slz1) complex, which catalyzes m⁶A methylation of mRNA. In budding yeast, this epitranscriptomic modification increases translation efficiency and is necessary for meiotic progression (Agarwala et al., 2012; Wang et al., 2015).

We measured *MUM2* expression by RT-qPCR during meiotic induction. Fourteen hours after transfer to sporulation medium, *MUM2* was still repressed in uncured [*SMAUG*⁺] cells (Figure 5D). However, 24 hours after transfer to sporulation medium, *MUM2* expression was comparable between in uncured and cured cells (Figure 5D). *SPS1*, a well-defined sporulation marker that lacks an SRE, was not differentially expressed at early timepoints. However, its levels were lower in uncured cells at a later time point, likely reflecting the delayed meiotic progression caused by the prion (Figure S4C; Chu et al., 1998).

The early reduction in *MUM2* expression in [*SMAUG*⁺] cells echoed the delayed sporulation linked to this prion. We tested whether enhanced *MUM2* expression could

counteract the delayed sporulation of [*SMAUG*⁺] cells, introducing [*SMAUG*⁺] into strains in which *MUM2* was expressed at its endogenous locus, either under the control of its native promoter or a strong constitutive promoter (*NOPI*; Yofe et al., 2016). [*SMAUG*⁺] cells expressing *MUM2* from its endogenous promoter sporulated poorly. By contrast, constitutive overproduction of *MUM2* rescued sporulation (Figure 5E).

We next investigated whether Vts1 binding to *MUM2* is necessary for [*SMAUG*⁺]’s impact on meiosis. We permuted two key nucleotides *MUM2*’s SRE using a CRISPR/Cas9 system (Anand, 2017; Aviv et al., 2006; Aviv et al., 2003; She et al., 2017). We then scored sporulation in cured and uncured strains harboring either this mutation or a wild type *MUM2* sequence. When *MUM2*’s SRE was mutated in uncured BY4743, sporulation efficiency increased significantly, and was similar to values we observed in cured strains (Figure 5F). By contrast, mutation *MUM2*’s SRE did not affect sporulation in cells that did not harbor the prion. Combined with our overexpression experiments, these data establish that [*SMAUG*⁺] controls meiotic commitment in large part via enhanced repression of *MUM2*.

Lastly, we asked if degradation of *MUM2* also mediated the mitotic growth advantage the observed in [*SMAUG*⁺] cells. If this were the case, deletion of *MUM2* in a cured strain, mimicking repression by [*SMAUG*⁺], should increase growth rate. Indeed, we observed a 1.5-fold increase in the growth rate of cured *mum2* cells (Figure S4D). *MUM2* overexpression had the opposite effect (Figure S4E). These data establish that changes in *MUM2* expression can account for the impact of [*SMAUG*⁺] on mitotic proliferation and meiotic differentiation alike.

Repeated occurrence of an element resembling [*SMAUG*⁺] in nature

Finally we investigated the breadth of [*SMAUG*⁺] in nature, examining 26 natural isolates from the *Saccharomyces* Genome Resequencing Project (Liti et al., 2009), collected from diverse ecological niches. The *URA3* gene has been deleted in these isolates (Cubillos et al., 2009), allowing us to cure them of prions by transient expression of a plasmid-borne dominant-negative Hsp70, as described above. Of the 26 strains, 21 yielded successful transformants, enabling us to obtain at least three independent curing events per isolate.

To assay Vts1 activity, we employed the plasmid-borne galactose-inducible *GFP-SRE* reporter construct described above. Nine of the 21 cured strains exhibited a significant increase in GFP fluorescence, ranging from 15% to 250% (Figure 6A, S5A and S5B). Eight of the remaining cured strains exhibited no significant change in GFP fluorescence (Figure S5C), and three exhibited very modest decreases (Figure S5D). We next transformed the nine cured isolates that exhibited an increase in fluorescence with a *GFP-SRE*-construct with abolished Vts1 binding. Eight of these strains showed no difference in *GFP-SRE*-fluorescence upon curing (Figure S6A). Thus, many wild *S. cerevisiae* strains harbor prion-like elements with the characteristics of [*SMAUG*⁺].

We next asked whether curing also affected the sporulation of these natural isolates. One strain, isolated from soil, exhibited no change in sporulation when cured (Figure S6B and S6C). Two others, isolated from barrel fermentation and grapes, did not sporulate within 48

hours, but did sporulate to the same degree after 120 hours (Figure S6C). However, five of the eight [*SMAUG*⁺] strains sporulated more efficiently after curing, ranging from a two- to seven-fold increase (Figure 6B and S6A). These data suggest that [*SMAUG*⁺], or a substantially equivalent element, arises commonly in both laboratory and natural isolates of *S. cerevisiae*, and exerts a strong influence on the meiotic developmental program.

Polymorphic [*SMAUG*⁺] variants in nature

The increased strength of the induced [*SMAUG*⁺] variant led us to speculate that this *prior* might form distinct, but stable, activity states, also known as ‘strains’ or ‘polymorphs’. Strain-to-strain variation in curing-dependent expression of the *GFP-SRE* reporter (Figure 6A) led us to further hypothesize that natural yeast isolates could harbor distinct conformational variants of [*SMAUG*⁺]. If true, these variants might template the Vts1 protein in different ways. First, we confirmed that lysates from the naturally [*SMAUG*⁺] BY4743 laboratory strain could seed the condensation of fluorescently-labeled, purified Vts1 protein (Figure 7A; Chakravarty et al., submitted; Figure S7A). In contrast, lysates from selectively cured BY4743 did not seed Vts1 condensates (Figure S7A).

Next, we tested whether lysates of natural yeast isolates exhibiting [*SMAUG*⁺]-like characteristics could also seed Vts1 condensation. Among these lysates, we observed a range of assembly properties. Lysates from strains isolated from oak bark (YPS606) and stingless bee (UW0PS05–227.2) did not promote formation of large condensates. These strains may either have slower assembly kinetics or express a *trans* factor that inhibits visible Vts1 condensate formation. Two lysates (from cactus and cactus fruit isolates; USOPS87–2421, USOPS83–787.3) formed condensates that closely resembled each other in size and intensity (Figure 7B). A third lysate (from palm flower nectar isolates; UW0PS03–461.4) seeded assemblies that resembled those generated from BY4743 lysate (Figure 7B and S9A). Lysates from DBVPG6765, a natural strain that did not exhibit a curing-dependent increase in *GFP-SRE* expression (‘natural control’), did not drive Vts1 condensation (Figure 7B).

Vts1 condensates are readily discernible due to their brightness relative to unassembled protein. As a proxy for condensate formation, we therefore measured the average pixel intensity in each sample. This value correlated with the degree of [*SMAUG*⁺]-mediated repression of *GFP-SRE* expression (Figure 7C; $p=0.006$), suggesting that the activity of a [*SMAUG*⁺] variant can be predicted by its patterns of self-assembly. *VTS1* transcript levels were similar among these natural strains; any modest differences did not correlate with seeding capacity or [*SMAUG*⁺]-driven phenotypes (Figure S7B). These data establish that natural isolates of *S. cerevisiae* not only harbor [*SMAUG*⁺], but also exhibit quantitative differences in the strength of [*SMAUG*⁺] traits. We conclude that [*SMAUG*⁺] exists as distinct variants in nature, tuning a fundamental cellular decision.

DISCUSSION

Adaptive anticipation of future environments has been theorized and observed in the laboratory (Mitchell et al., 2009). Yet, our understanding of the molecular mechanisms that govern such behavior in nature remains limited. Using *S. cerevisiae* as a model, we took

advantage of the nutrient dependence of gametogenesis to simulate adaptive prediction in fluctuating environments. These simulations suggested that altering the sensitivity of relationship between starvation and meiosis, via an epigenetic mechanism that is heritable yet reversible, would offer significant adaptive benefit. Guided by these inferences, we identified a heritable prion conformation of an evolutionary ancient RNA binding protein, [*SMAUG*⁺], that meets these criteria. This prion provides a mechanism for capturing information about the quality of environmental fluctuations and transmitting it to future generations, adaptively modulating the cell fate decisions between mitosis and gametogenesis.

This form of epigenetic regulation is pervasive. [*SMAUG*⁺] occurs in most laboratory strains of *S. cerevisiae*, in which common husbandry conditions would favor its enrichment. [*SMAUG*⁺] is also common in natural *S. cerevisiae* isolates from diverse ecological niches, where it strongly impacts sporulation efficiency. Thus, [*SMAUG*⁺] may have repeatedly influenced *S. cerevisiae*'s ability to adapt to new environments. The absence of [*SMAUG*⁺] in most SGRP strains suggests that the limited handling of these strains has not itself induced [*SMAUG*⁺]. These discoveries force a reevaluation of the prevalence and importance of prions. Once thought to be obscure exceptions to the central dogma, these heritable protein conformations may in fact be common in biology, capable of adaptively modulating developmental strategies.

Due to its ubiquity in *S. cerevisiae*, [*SMAUG*⁺] is a major contributor to variation in sporulation observed across this species. Our findings have strong implications for functional genomics: between *S. cerevisiae* strains with a degree of polymorphism similar to two humans, up to ~38% of phenotypic variance is unexplained by genetics (Bloom et al., 2015). [*SMAUG*⁺] highlights the strong influence that unexplored forms of epigenetics can have on phenotypes of fundamental biological importance. Indeed, [*SMAUG*⁺] potential interactions between [*SMAUG*⁺] and other types of protein aggregates that regulate meiosis (e.g. Rim4) raise exciting questions for future study.

The well-studied prion [*PSI*⁺] can exist as multiple stable conformational variants, each with a different phenotypic strength (Bateman and Wickner, 2013; Tanaka et al., 2004; Tanaka et al., 2006; Toyama et al., 2007). However, because 'wild' [*PSI*⁺] variants are of similar strengths (Halfmann et al., 2012), it has remained unknown whether such variation is exploited in nature. Here, we found that [*SMAUG*⁺] adopts multiple functionally distinct variants in the wild. Given the adaptive advantage provided by the prion, these variants could be fine-tuned to the frequency of fluctuations inherent to a niche. Together, along with the hyperactivity of [*SMAUG*⁺] (Chakravarty et al., submitted), these data highlight an emergent dimension of protein structure and function has been largely unappreciated to date, and may be regularly utilized in nature.

In *Drosophila*, the homolog of Vts1, Smaug, is essential for early development, governing the degradation of transcripts during maternal-to-zygotic transition (Tadros et al., 2007). Here we show that Vts1 controls another developmental process, gametogenesis, in *S. cerevisiae*. The natural [*SMAUG*⁺] prion degrades its target transcripts at an elevated rate. Our findings reveal a key biological role for this hyperactivity: repression of transcripts

involved early meiosis. The ensuing delay in sporulation is mediated by degradation of the message encoding the mRNA methylase subunit *MUM2*, a target of Vts1, and itself a critical post-transcriptional regulatory node in meiosis (Agarwala et al., 2012). More than 300 other SREs have been identified in yeast, including in genes unrelated to meiosis (She et al., 2017). Thus, sporulation could be one of many phenotypes regulated by [*SMAUG*⁺].

Prion induction and loss is often influenced by the environment (reviewed in Harvey et al., 2018). Based on theory (Jablonka et al., 1995; Kussel and Leibler, 2005), one would predict that [*SMAUG*⁺] could be induced, in a quasi-Lamarckian fashion, by the same conditions in which it is advantageous. Notably in this regard, across the thousands of conditions in which gene expression has been measured in yeast, *VTS1* is most upregulated in ‘return to growth’ assays – conditions when *S. cerevisiae* cells experience nutrient replenishment before irreversibly committing to meiosis (Friedlander et al., 2006; Figure 5A). This natural upregulation would be expected to induce [*SMAUG*⁺] (by virtue of mass action) as a function of the reproducibility of the environmental fluctuation: as the population experiences regular cycles of nutrient replenishment, more cells would convert to and benefit from [*SMAUG*⁺]. Indeed, exogenous upregulation is regularly used as a means of robustly inducing prion formation (Chakrabortee et al., 2016; Masison and Wickner, 1995; Wickner, 1994; Wickner et al., 2006). Characterizing how rates of [*SMAUG*⁺] gain and loss are tuned in different fluctuating environments represents an exciting avenue for future research.

In sum, we have demonstrated that the [*SMAUG*⁺] prion, pervasive in both nature and the laboratory, serves as a ubiquitous means of adaptive transgenerational memory in *S. cerevisiae*. [*SMAUG*⁺] allows *S. cerevisiae* cells to make ‘wagers’ that were beneficial to their ancestors to anticipate future environmental fluctuations, substantially influencing their growth and differentiation strategies. In addition, this prion regularly explores its conformational space in natural populations, encoding distinct stable polymorphs that amplify the phenotypic diversification sparked by this protein-based genetic element. [*SMAUG*⁺] controls some of the best studied traits in one of the most widely used model organisms in experimental science. It is present in genetic backgrounds that have been subject to thousands of experiments by laboratories around the world. Yet targeted and ‘omics’ technologies have been blind to its presence and importance. Together our findings portend a much larger and mechanistically diverse ‘prionome’ that is hidden in plain sight, and remains to be unveiled.

STAR METHODS

LEAD CONTACT AND MATERIALS AVAILABILITY

Correspondence and requests for materials should be addressed to Lead Contact Daniel F. Jarosz (danjarosz.aa@gmail.com). All unique reagents generated in this study are available from the Lead Contact without restriction.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

S. cerevisiae strains were obtained from the sources indicated (Key Resources Table). Natural *S. cerevisiae* isolates were obtained from the *Saccharomyces* Genome Resequencing

Project (SGRP) Strain Set 2 and used as founder strains (Cubillos et al., 2009; <https://catalogue.ncyc.co.uk/sgrp-sets>). These strains have *URA3* deleted.

All *S. cerevisiae* strains were stored as glycerol stocks at -80°C . Before use, strains were revived on YPD. Cultures were grown at 30°C . For growth measurements, cells were inoculated from solid YPD into complete synthetic media containing 2% galactose for pre-growth. Cells were then normalized to $\text{OD}_{600} = 0.1$ and $1\ \mu\text{l}$ was inoculated into the media of interest.

For plasmid transformations, a standard lithium-acetate protocol was used (Gietz et al., 1992). Cells were inoculated into YPD and grown to saturation overnight. Cells were harvested, washed in sterile water, and suspended in a transformation mix ($120\ \mu\text{l}$ of 50% w/v PEG 3500, $17\ \mu\text{l}$ of 1M lithium acetate, $24\ \mu\text{l}$ of boiled salmon sperm carrier DNA (2mg/ml), and $17\ \mu\text{l}$ of H_2O containing up to $1\ \mu\text{g}$ of plasmid). Cells were incubated at 30°C for 30 minutes, and then at 42°C for 20 minutes, before washing, pelleting, and plating on selective media.

Integration transformations were performed using a standard electroporation protocol (Thompson et al., 1998). Cells were inoculated from solid media into 5 ml of YPD and grown to saturation overnight. The culture was then diluted in 25 ml of YPD and grown to an OD_{600} of ~ 0.5 . Cells were then pelleted and suspended in 9 ml of TE buffer and 1 ml of 1 M lithium acetate, and incubated for 45 min at 30°C , mixed with $250\ \mu\text{l}$ of 1 M DTT was added, and cells were incubated for an additional 15 min. Cells were then washed twice with water and once with sorbitol, and then resuspended in $60\ \mu\text{l}$ of cold 1 M sorbitol. Salmon sperm DNA ($1.7\ \mu\text{l}$) and cassette DNA ($1\ \mu\text{g}$ total) was added to $40\ \mu\text{l}$ of cell mixture, and the mixture was then subjected to electroporation ($0.2\ \text{cm}$ gap cuvette, $1.5\ \text{kV}$, $24\ \mu\text{F}$, $200\ \text{ohm}$). Cells were recovered overnight in YPD before plating on selective medium.

METHOD DETAILS

Modeling—In a nutrient-rich environment t_1 , both populations undergo exponential growth:

$$x_{\text{sporulator}} = x_{\text{sporulator, initial}} e^{\mu_{\text{sporulator}} t_1} \quad (1)$$

$$x_{\text{proliferator}} = x_{\text{proliferator, initial}} e^{\mu_{\text{proliferator}} t_1} \quad (2)$$

$x_{\text{sporulator}}$ is the fraction of the population that are sporulators; $x_{\text{proliferator}}$ is the fraction of the population that are proliferators. For the first cycle ($N = 1$), the initial fraction is always 0.5 for each developmental strategy, unless otherwise specified. $\mu_{\text{sporulator}}$ and $\mu_{\text{proliferator}}$ are the growth rates for proliferators and sporulators, respectively.

In a nutrient-poor environment, t_2 , both populations enter the sporulation program. The fraction of the population that form stress-resistant spores is determined by a Hill function with respect of time in nutrient poor environments. A Hill function allows different strains to

have different sporulation kinetics while maintaining the same maximum sporulation efficiency – two features we observed in experiments (Figure 2B).

$$spores_{sporulator} = x_{sporulator} \frac{k_2^{sporulator} t_2}{k_1^{sporulator} k_2^{sporulator} + t_2 k_2^{sporulator}} \quad (3)$$

$$spores_{proliferator} = x_{proliferator} \frac{k_2^{proliferator} t_2}{k_1^{proliferator} k_2^{proliferator} + t_2 k_2^{proliferator}} \quad (4)$$

$spores_{sporulator}$ and $spores_{proliferator}$ are the fractions of the sporulator and proliferators populations, respectively, populations that form spores. k_1 and k_2 are coefficients obtained by fitting a Hill function to the sporulation dynamics observed in Figure 2B.

We define the survival probabilities during the die-off event as being equal for the haploids of sporulators and proliferators.

$$x_{sporulator} = p_{spores} spores_{sporulator} + p_{diploids} (x_{sporulator} - spores_{sporulator}) \quad (5)$$

$$x_{proliferator} = p_{spores} spores_{proliferator} + p_{diploids} (x_{proliferator} - spores_{proliferator}) \quad (6)$$

After the die-off event, we are left with the resulting populations of individuals for each developmental strategy. This is the starting population for the subsequent cycle. The cycle continues for N nutrient rich, nutrient poor, and die-off phases. For all plots, unless otherwise specified, the simulation was run for $N = 10$ cycles.

For physiological parameter estimates we used the following observations: 1) relative growth rates for advantageous gene deletions can be as large as $\mu \cong 1.1$ (Breslow et al., 2008); 2) spores can be ~ 100-fold more resistant to environmental stressors relative to unsporulated diploids (Coluccio et al., 2008); 3) in populations of *S. cerevisiae*, sporulation occurs over tens to hundreds of hours (Gerke et al., 2006; Mortimer, 2000).

Curing—Strains were cured by transforming an *URA3*-selectable 2 μ plasmid encoding a dominant-negative HSP70 (Ssa1-K69M; Chakrabortee et al., 2016; Garcia et al., 2016; Lagaudriere-Gesbert et al., 2002) under the control of a constitutive promoter (*pGPD*). Transformants were passaged on selective media five times to allow growth of single colonies. Next, transformants were passaged three times on non-selective media (YPD) to allow for plasmid loss, which was confirmed by the lack of growth on selective media (SD-Ura).

Cytoduction was performed as described previously (Chakrabortee et al., 2016) Strains harboring the *kar1-15* allele, which are deficient in nuclear fusion, were converted to petites

by growing in YPD + 0.25% ethidium bromide, and then recovered on YPD agar. Respiration incompetence was confirmed by lack of growth on YP-glycerol. Cured and uncured BY4741 (donor strains) were mated to a cured α strain harboring the *kar1-15* allele (recipient strain). Heterokaryons were isolated by selecting for strains that were respiration competent and for a *MATa* auxotrophic marker. Cytoeductants were passaged again on SD-Met, and then validated to be haploid. Reverse cytoeductions were performed by mating the above cytoeductants to cured BY4741 petites, and then selected for mitochondrial respiration and auxotrophic growth on lysine, and validated to be haploids. These cytoeductants were mated to cured strains to generate diploids.

Sporulation phenotyping—Strains were inoculated from single colonies on fresh plates into pre-sporulation media (0.8% Yeast extract, 0.3% Bacto-peptone, 0.01% adenine sulfate, 10% dextrose) and grown with rotation at 30°C for 48 hours. At this point, cells were spun down at 1500 *g* for 3 minutes, washed in H₂O, pelleted, and resuspended in sporulation media (1% potassium acetate, 0.1% yeast extract, 0.05% dextrose, 0.01% amino acid add-back (1:1:5 uracil, histidine, leucine). Cultures were kept on a rotating wheel at 25°C. Unless otherwise specified, cultures were sporulated for 120 hours. DIC imaging was performed at a magnification of 63× on a Leica DMI6000 microscope; two to three images were acquired per sample. For each image, ~100 cells were counted, and spores were scored to calculate the sporulated fraction.

For natural isolates, growth and sporulation was performed in 96 well plates. Plates were Aero sealed and shaken at 500 rpm. Sporulation was scored after 48 hours unless otherwise specified. All other conditions were identical to those for cells sporulated in 5 ml culture tubes (as described above).

RT-qPCR—Five cultures were grown and transferred to sporulation media. At each time point, 1 ml of culture was pelleted and washed with H₂O, and pelleted again before flash freezing. RNA was extracted using a standard phenol–chloroform procedure (Collart and Oliviero, 2001). RNA concentration was normalized based on 260 nm/280 nm ratios, and then used to synthesize cDNA using oligo-T primers. qPCR was performed using KAPA SYBR FAST qPCR master mix (Kapa Biosystems). Primers against the housekeeping gene TAF10 were used as controls for relative quantification (Teste et al., 2009). Primer sequences used for probing *MUM2* and *SPS1* are listed in the Key Resources Table. For measurement of relative *VTS1* levels across wild strains, 5 ml of mid-exponential cultures were processed through a near-identical workflow. Primers used for probing relative *VTS1* abundance are listed in the Key Resources Table

Library preparation, RNA sequencing, and analyses—For all samples, RNA was extracted from diploid yeast strains (BY4743 and BY4743 selectively cured of [*SMAUG*⁺]; two biological replicates at each time point for each strain) before (0 h) and after initiation of the sporulation program (14 h) using a standard phenol–chloroform protocol. Libraries were generated using standard kits (NEBNext® Ultra™ II RNA library prep kit Cat# E7770S, poly-A enrichment). Further quality control and quantification was performed by analysis on an Agilent 2100 Bioanalyzer and RT-qPCR (SYBR staining). All samples were sequenced to a read depth of ~10,000,000 (1 × 75 bp) on a single flow cell of an Illumina

NextSeq 550™. Quality control of reads was performed using FastQC (Babraham Institute). Reads were de-duplicated and pseudo-aligned against the *Saccharomyces cerevisiae* strain S288C reference genome assembly R64, and then transcript-level quantification was performed using Kallisto. Differential expression analyses were performed using the DESeq2 package in R. Principal component analyses of most differentially regulated genes and significantly altered transcripts were performed using default settings in the DESeq2 package, which employs the Benjamini-Hochberg approach for approximating the false discovery rate (FDR). RNA-seq data is deposited into Gene Expression Omnibus (GEO; GSE138559).

Protein transformation—Mid-exponential cultures of selectively cured [*smaug*[−]] haploid strains were transformed with Vts1 condensates generated from purified protein or with BSA (as a control), following a workflow similar to that described in the accompanying manuscript (Chakravarty et al. submitted). In brief, cell pellets were washed and spheroplasted with Zymolyase®–100T (Sunrise Science Products, Cat# 0766555) in SCE buffer (1 M sorbitol, 10 mM EDTA, 10 mM DTT, 100 mM Na-citrate pH 5.8). Following exposure to protein of interest and a co-transformed *LEU-selectable* plasmid, these spheroplasts were collected and resuspended in 250 µl of SOS buffer (1 M sorbitol, 7 mM CaCl₂, 0.25% yeast extract, 0.5% Bacto-peptone). To avoid disrupting the cell membrane, all manipulations were performed using cut pipette tips with widened apertures. This mixture was incubated at 30°C for 3 h, after which these cells were plated on solid medium (SD-Leu) that was supplemented with 1.2 M sorbitol. Following plating, these cells were overlaid with soft agar (0.8% agar) of an otherwise identical composition, and the plates were incubated at 30°C for 3–5 days. Individual transformants for both sets of transformations were picked and passaged on SD-Leu media (for ~75 generations), mated to a cured strain of opposite mating type, and then subjected to sporulation measurements using workflows described above.

Yeast cell lysate extraction and seeding—Cells from yeast strains in the SGRP collection were harvested from 100 ml cultures in mid-exponential phase by centrifuging at 5000 *g* for 2 min at 4°C. All subsequent steps were performed at or below 4°C, as stated, and the method for native yeast cell lysis was nearly identical to that described in the accompanying manuscript (Chakravarty et al. 2019). In brief, cells were washed twice with wash buffer (50 mM HEPES-NaOH [pH 7.6], 2 mM EDTA, 0.8 M sorbitol, 300 mM sodium glutamate, 3 mM DTT added fresh immediately before use) and resuspended in ½ packed cell volume of lysis buffer (100 mM HEPES-NaOH [pH 7.6], 0.8 M sorbitol, 950 mM sodium glutamate, 10 mM magnesium acetate, 5 mM DTT, and one Complete Protease Inhibitor Cocktail tablet (Roche Cat# 11836145001; per 5 ml) added fresh immediately before use). This resuspensate was then frozen dropwise in liquid nitrogen, and the resultant beads were stored at −80°C until needed. Yeast cell beads were loaded into a steel vial pre-chilled at −80°C and processed using a CryoMill (Retsch) using the following program sequence: 1.5 min precooling at 5 Hz, 9 cycles of 2 min each at 15 Hz, 30 sec of gap at 5 Hz between each cycle. Next, lysis buffer was added to the mixture resulting from thawing the powder in a chilled conical tube (1/3rd of the total volume), and the sample was transferred to an Eppendorf tube. This lysate was then centrifuged to remove cell debris, and the

resultant supernatant was dialyzed (using a 10 kDa MWCO membrane) against dialysis buffer (50 mM HEPES-NaOH [pH 7.6], 2 mM EDTA, 10 % (v/v) glycerol, 300 mM sodium glutamate, 5 mM magnesium acetate, 3 mM DTT added fresh immediately before use). Total protein concentration was measured by A₅₉₅ using the Bradford assay reagent, and aliquots of the lysates were stored at -80°C. *In vitro* seeding experiments using these lysates were performed at room temperature for 4 h; the molar ratio of Vts1 from lysate to purified Vts1 was ~1:163 (Ghaemmaghami et al., 2003). Imaging was performed using standard epifluorescence microscopy (Leica DMI6000) using Cy3 filter blocks.

Competition assays—Strains were transformed using an integrating plasmid that incorporated a fluorescent protein (either mNeonGreen [Neon] or mKate2 [Kate]) under the control of a strong constitutive promoter (*TDH3*) and a hygromycin-selectable marker into the *HO* locus (Wong et al., 2018; Zalatan et al., 2012). Transformants were selected on YPD + 200 µg/L hygromycin B. To check for proper integration of the integration cassette, PCR was performed on hygromycin-resistant transformants using a cassette nested primer and a flanking primer hybridizing within the *HO* locus. Fluorescent protein expression was checked on a confocal microscope (mNeonGreen: excitation, 450–490 nm, emission, 500–550 nm; mKate2, excitation 630–640 nm, emission, 690–750 nm). Growth curves of each PCR-confirmed, fluorescent transformant were generated to ensure that exogenous fluorescent protein expression did not result in an obvious growth defect.

Neon- and Kate-expressing strains were pre-grown for 48 hours in complete synthetic media containing 2% galactose and 200 µg/ml hygromycin B. Strains were diluted to an OD of 0.1. Competitions were carried out by mixing a Neon strain and a Kate strain in a 1:1 ratio, and inoculating 1 µl of this mixture into 150 µl of media of interest. Inoculated cultures were grown at 30°C for 24 or 48 hours. At this point, they were diluted to an OD of 0.1 using H₂O. One microliter of this diluted culture was used to inoculate fresh media for the subsequent growth step. The remainder of the diluted culture was fixed using 4% paraformaldehyde for 15 minutes and stored in 1.2 M sorbitol + 0.1 M potassium phosphate at 4°C until flow cytometry analysis. Flow cytometry was performed on a BD LSR II, exciting at 488 nm for Neon and 561 nm for Kate. A total of 10,000 events per sample was collected. Cultures were passaged 5 times total.

To correct for any tag-specific growth effect, control competitions containing the same strains marked with different fluorescent proteins (i.e. cured BY4743 expressing Kate competed with cured BY4743 expressing Neon) were also performed. Any difference in growth observed in these competitions was treated as a tag-specific growth effect and normalized out in analyses of strain-specific growth differences. Selection coefficient was calculated from the slope of a line fitted to the natural log-transformed data (Chevin, 2011).

Vts1 activity—To assay Vts1 activity, we used a construct a galactose-inducible GFP followed by three tandem SREs. In the permuted version, the three tandem SREs were mutated to abolish Vts1 binding. Cells containing the construct were pre-grown in dropout media with raffinose as the carbon source. For BY4743, we used a *GFP-SRE* construct with a *HIS3* selectable marker. For the SGRP collection, we used a *GFP-SRE* construct with a *URA3* marker. After pre-growth for 48 hours, cultures were diluted to an OD of 0.1 and

used to inoculate new cultures in dropout S-raffinose + 0.2% galactose to induce expression of *GFP-3xSRE*. Cells were grown to mid-exponential phase (OD = 0.5; ~12 h). Cells were imaged using a Leica inverted fluorescence microscope with a Hamamatsu Orca 4.0 camera. (GFP excitation: 450–490 nm; emission: 500–550 nm; software: LASX DMI6000B; refraction index: 1.518; aperture: 1.4; exposure time: 750 ms). DIC images of the same fields of view were also collected to define cell edges.

CRISPR/Cas9 mutation—We followed a protocol from the Haber laboratory (Anand, 2017). The guide DNA (TTATGATCCCCAACATTCGT) was designed in to target base pairs 21–40 of the *MUM2* coding sequence, upstream of an endogenous PAM sequence, TGG. A homology template that introduced 5 synonymous changes to the guide DNA sequence (CTACGACCCCCAGCATTCCT) and either the wild-type or permuted SRE element was used.

Automated GFP fluorescence quantification—All analyses were performed using established pipelines in CellProfiler. Using the DIC image, cells were defined using the Sobel edge-finding method. Cells that did not have a form factor ($4\pi \times \text{area}/\text{perimeter}^2$) greater than 0.5 and an area between 2000 and 10000 pixels (i.e., cells that were unsuccessfully recognized) were ignored. Next, integrated GFP fluorescence intensity was calculated for each successfully recognized cell.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification and accompanying statistical tests for all experiments are described in the Results section and figure legends. The Mann-Whitney *U* test was used to compare measurements between two samples. When the means of more than two samples were being compared, a one-way test of variance was used followed by Sidak's multiple comparison test. Fisher's exact tests were used to compare overlap between sets of proteins and genes). *p*-values < 0.05 were interpreted as reflecting significant differences.

DATA AND CODE AVAILABILITY

All gene expression data collected are deposited in Gene Expression Omnibus (GEO) under accession number GSE138559. Representative microscopy images have been submitted to Mendeley (10.17632/kkst3822v4.1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Drs. C. Smibert and Mo Khalil who generously provided bacterial strains for this study. We also thank Drs. S. Boeynaems, L. Xie, Z. Harvey, C. Jakobson, and members of the Jarosz laboratory for materials, discussions, and/or critical reading of the manuscript. Soledad Larios was critical in ensuring a constant supply of sterilized laboratory-ware. Flow cytometry analysis for this project was done on instruments in the Stanford Shared FACS Facility. This work was supported by National Institutes of Health (NIH) Grants DP2-GM119140 (to D.F.J.). A.K.I. was supported by a National Institutes of Health (NIH) Cell and Molecular Biology Training Grant (5T32GM 7276–42). A.K.C. was supported as a Howard Hughes Medical Institute fellow of the Damon Runyon Cancer Research Foundation (DRG2221–15) and by an NIH Pathway to independence award (1K99GM128180–

01). D.F.J. was also supported as a Searle Scholar, as a Kimmel Scholar, as a Vallee Scholar, by a Science and Engineering Fellowship from the David and Lucile Packard Foundation, and by a Career Award (1453762) from the National Science Foundation (NSF).

REFERENCES

- Agarwala SD, Blitzblau HG, Hochwagen A, and Fink GR (2012). RNA methylation by the MIS complex regulates a cell fate decision in yeast. *PLoS Genet* 8, e1002732. [PubMed: 22685417]
- Alberti S, Gitler AD, and Lindquist S (2007). A suite of Gateway cloning vectors for high-throughput genetic analysis in *Saccharomyces cerevisiae*. *Yeast* 24, 913–919. [PubMed: 17583893]
- Anand R, Memisoglu G, Haber J, (2017). Cas9-mediated gene editing in *Saccharomyces cerevisiae*. Protocol Exchange.
- Aviv T, Lin Z, Ben-Ari G, Smibert CA, and Sicheri F (2006). Sequence-specific recognition of RNA hairpins by the SAM domain of Vts1p. *Nat Struct Mol Biol* 13, 168–176. [PubMed: 16429151]
- Aviv T, Lin Z, Lau S, Rendl LM, Sicheri F, and Smibert CA (2003). The RNA-binding SAM domain of Smaug defines a new family of post-transcriptional regulators. *Nat Struct Biol* 10, 614–621. [PubMed: 12858164]
- Bateman DA, and Wickner RB (2013). The [PSI⁺] prion exists as a dynamic cloud of variants. *PLoS Genet* 9, e1003257. [PubMed: 23382698]
- Bell G, and Collins S (2008). Adaptation, extinction and global change. *Evol Appl* 1, 3–16. [PubMed: 25567487]
- Ben-Ari G, Zenvirth D, Sherman A, David L, Klutstein M, Lavi U, Hillel J, and Simchen G (2006). Four linked genes participate in controlling sporulation efficiency in budding yeast. *PLoS Genet* 2, e195. [PubMed: 17112318]
- Berchowitz LE, Kabachinski G, Walker MR, Carlile TM, Gilbert WV, Schwartz TU, and Amon A (2015). Regulated Formation of an Amyloid-like Translational Repressor Governs Gametogenesis. *Cell* 163, 406–418. [PubMed: 26411291]
- Bloom JS, Kotenko I, Sadhu MJ, Treusch S, Albert FW, and Kruglyak L (2015). Genetic interactions contribute less than additive effects to quantitative trait variation in yeast. *Nat Commun* 6, 8712. [PubMed: 26537231]
- Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, and Boeke JD (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14, 115–132. [PubMed: 9483801]
- Breslow DK, Cameron DM, Collins SR, Schuldiner M, Stewart-Ornstein J, Newman HW, Braun S, Madhani HD, Krogan NJ, and Weissman JS (2008). A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. *Nat Methods* 5, 711–718. [PubMed: 18622397]
- Calahan D, Dunham M, DeSevo C, and Koshland DE (2011). Genetic analysis of desiccation tolerance in *Saccharomyces cerevisiae*. *Genetics* 189, 507–519. [PubMed: 21840858]
- Caudron F, and Barral Y (2013). A super-assembly of Whi3 encodes memory of deceptive encounters by single cells during yeast courtship. *Cell* 155, 1244–1257. [PubMed: 24315096]
- Chakrabortee S, Byers JS, Jones S, Garcia DM, Bhullar B, Chang A, She R, Lee L, Fremin B, Lindquist S, et al. (2016). Intrinsically Disordered Proteins Drive Emergence and Inheritance of Biological Traits. *Cell* 167, 369–381 e312. [PubMed: 27693355]
- Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, and Lieberman SW (1995). Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi⁺]. *Science* 268, 880–884. [PubMed: 7754373]
- Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, Christie KR, Costanzo MC, Dwight SS, Engel SR, et al. (2012). *Saccharomyces* Genome Database: the genomics resource of budding yeast. *Nucleic Acids Res* 40, D700–705. [PubMed: 22110037]
- Chevin LM (2011). On measuring selection in experimental evolution. *Biol Lett* 7, 210–213. [PubMed: 20810425]
- Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, and Herskowitz I (1998). The transcriptional program of sporulation in budding yeast. *Science* 282, 699–705. [PubMed: 9784122]

- Collart MA, and Oliviero S (2001). Preparation of yeast RNA. *Curr Protoc Mol Biol* Chapter 13, Unit13 12.
- Coluccio AE, Rodriguez RK, Kernan MJ, and Neiman AM (2008). The yeast spore wall enables spores to survive passage through the digestive tract of *Drosophila*. *PLoS One* 3, e2873. [PubMed: 18682732]
- Conde J, and Fink GR (1976). A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc Natl Acad Sci U S A* 73, 3651–3655. [PubMed: 790391]
- Costanzo M, VanderSluis B, Koch EN, Baryshnikova A, Pons C, Tan G, Wang W, Usaj M, Hanchard J, Lee SD, et al. (2016). A global genetic interaction network maps a wiring diagram of cellular function. *Science* 353.
- Cubillos FA, Louis EJ, and Liti G (2009). Generation of a large set of genetically tractable haploid and diploid *Saccharomyces* strains. *FEMS Yeast Res* 9, 1217–1225. [PubMed: 19840116]
- Eaglestone SS, Ruddock LW, Cox BS, and Tuite MF (2000). Guanidine hydrochloride blocks a critical step in the propagation of the prion-like determinant [PSI(+)] of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 97, 240–244. [PubMed: 10618402]
- Franzmann TM, Jahnle M, Pozniakovskiy A, Mahamid J, Holehouse AS, Nuske E, Richter D, Baumeister W, Grill SW, Pappu RV, et al. (2018). Phase separation of a yeast prion protein promotes cellular fitness. *Science* 359.
- Garcia DM, Dietrich D, Clardy J, and Jarosz DF (2016). A common bacterial metabolite elicits prion-based bypass of glucose repression. *Elife* 5.
- Gerke J, Lorenz K, and Cohen B (2009). Genetic interactions between transcription factors cause natural variation in yeast. *Science* 323, 498–501. [PubMed: 19164747]
- Gerke JP, Chen CT, and Cohen BA (2006). Natural isolates of *Saccharomyces cerevisiae* display complex genetic variation in sporulation efficiency. *Genetics* 174, 985–997. [PubMed: 16951083]
- Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, and Weissman JS (2003). Global analysis of protein expression in yeast. *Nature* 425, 737–741. [PubMed: 14562106]
- Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418, 387–391. [PubMed: 12140549]
- Gietz D, St Jean A, Woods RA, and Schiestl RH (1992). Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res* 20, 1425. [PubMed: 1561104]
- Halfmann R, Alberti S, and Lindquist S (2010). Prions, protein homeostasis, and phenotypic diversity. *Trends Cell Biol* 20, 125–133. [PubMed: 20071174]
- Halfmann R, Jarosz DF, Jones SK, Chang A, Lancaster AK, and Lindquist S (2012). Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 482, 363–368. [PubMed: 22337056]
- Halfmann R, and Lindquist S (2010). Epigenetics in the extreme: prions and the inheritance of environmentally acquired traits. *Science* 330, 629–632. [PubMed: 21030648]
- Harvey ZH, Chen Y, and Jarosz DF (2018). Protein-Based Inheritance: Epigenetics beyond the Chromosome. *Mol Cell* 69, 195–202. [PubMed: 29153393]
- Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, and O'Shea EK (2003). Global analysis of protein localization in budding yeast. *Nature* 425, 686–691. [PubMed: 14562095]
- Itakura AK, Futia RA, and Jarosz DF (2018). It Pays To Be in Phase. *Biochemistry* 57, 2520–2529. [PubMed: 29509425]
- Jablonka E, Orborny B, Morlnar I, Kisdi É, Hofbauer J, and Czárán T (1995). The adaptive advantage of phenotypic memory in changing environments. *Phil Trans R Soc Lond B* 350, 133–141. [PubMed: 8577857]
- Jakobson CM, and Jarosz DF (2019). Molecular Origins of Complex Heritability in Natural Genotype-to-Phenotype Relationships. *Cell Syst* 8, 363–379 e363. [PubMed: 31054809]
- Jarosz DF, Brown JCS, Walker GA, Datta MS, Ung WL, Lancaster AK, Rotem A, Chang A, Newby GA, Weitz DA, et al. (2014a). Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism. *Cell* 158, 1083–1093. [PubMed: 25171409]

- Jarosz DF, Lancaster AK, Brown JCS, and Lindquist S (2014b). An evolutionarily conserved prion-like element converts wild fungi from metabolic specialists to generalists. *Cell* 158, 1072–1082. [PubMed: 25171408]
- Kamentsky L, Jones TR, Fraser A, Bray MA, Logan DJ, Madden KL, Ljosa V, Rueden C, Eliceiri KW, and Carpenter AE (2011). Improved structure, function and compatibility for CellProfiler: modular high-throughput image analysis software. *Bioinformatics* 27, 1179–1180. [PubMed: 21349861]
- King OD, and Mase J (2007). The evolution of bet-hedging adaptations to rare scenarios. *Theor Popul Biol* 72, 560–575. [PubMed: 17915273]
- Kussell E, and Leibler S (2005). Phenotypic Diversity, Population Growth, and Information in Fluctuating Environments. *Science* 309, 2075. [PubMed: 16123265]
- Lachmann M, and Jablonka E (1996). The inheritance of phenotypes: an adaptation to fluctuating environments. *J Theor Biol* 181, 1–9. [PubMed: 8796186]
- Lagaudriere-Gesbert C, Newmyer SL, Gregers TF, Bakke O, and Ploegh HL (2002). Uncoating ATPase Hsc70 is recruited by invariant chain and controls the size of endocytic compartments. *Proceedings of the National Academy of Sciences of the United States of America* 99, 1515–1520. [PubMed: 11818572]
- Lancaster AK, Bardill JP, True HL, and Mase J (2010). The spontaneous appearance rate of the yeast prion [PSI⁺] and its implications for the evolution of the evolvability properties of the [PSI⁺] system. *Genetics* 184, 393–400. [PubMed: 19917766]
- Lancaster AK, and Mase J (2009). The evolution of reversible switches in the presence of irreversible mimics. *Evolution* 63, 2350–2362. [PubMed: 19486147]
- Lang GI, and Murray AW (2008). Estimating the per-base-pair mutation rate in the yeast *Saccharomyces cerevisiae*. *Genetics* 178, 67–82. [PubMed: 18202359]
- Lindquist S, Patino MM, Chernoff YO, Kowal AS, Singer MA, Liebman SW, Lee KH, and Blake T (1995). The role of Hsp104 in stress tolerance and [PSI⁺] propagation in *Saccharomyces cerevisiae*. *Cold Spring Harb Symp Quant Biol* 60, 451–460. [PubMed: 8824419]
- Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, Davey RP, Roberts IN, Burt A, Koufopanou V, et al. (2009). Population genomics of domestic and wild yeasts. *Nature* 458, 337–341. [PubMed: 19212322]
- Love MI, Huber W, and Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, 550. [PubMed: 25516281]
- Marston AL, and Amon A (2004). Meiosis: cell-cycle controls shuffle and deal. *Nat Rev Mol Cell Biol* 5, 983–997. [PubMed: 15573136]
- Masison DC, Kirkland PA, and Sharma D (2009). Influence of Hsp70s and their regulators on yeast prion propagation. *Prion* 3, 65–73. [PubMed: 19556854]
- Masison DC, and Wickner RB (1995). Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. *Science* 270, 93–95. [PubMed: 7569955]
- McDonald MJ, Rice DP, and Desai MM (2016). Sex speeds adaptation by altering the dynamics of molecular evolution. *Nature* 531, 233–236. [PubMed: 26909573]
- Miller MP, Amon A, and Unal E (2013). Meiosis I: when chromosomes undergo extreme makeover. *Current Opinion in Cell Biology* 25, 687–696. [PubMed: 23916768]
- Mitchell A, Romano GH, Groisman B, Yona A, Dekel E, Kupiec M, Dahan O, and Pilpel Y (2009). Adaptive prediction of environmental changes by microorganisms. *Nature* 460, 220–224. [PubMed: 19536156]
- Mortimer RK (2000). Evolution and variation of the yeast (*Saccharomyces*) genome. *Genome Res* 10, 403–409. [PubMed: 10779481]
- Neiman AM (2011). Sporulation in the budding yeast *Saccharomyces cerevisiae*. *Genetics* 189, 737–765. [PubMed: 22084423]
- Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, Hwang SY, Davis RW, and Esposito RE (2000). The core meiotic transcriptome in budding yeasts. *Nat Genet* 26, 415–423. [PubMed: 11101837]
- Rando OJ, and Verstrepen KJ (2007). Timescales of genetic and epigenetic inheritance. *Cell* 128, 655–668. [PubMed: 17320504]

- Saad S, Cereghetti G, Feng Y, Picotti P, Peter M, and Dechant R (2017). Reversible protein aggregation is a protective mechanism to ensure cell cycle restart after stress. *Nat Cell Biol* 19, 1202–1213. [PubMed: 28846094]
- Saarikangas J, and Barral Y (2015). Protein aggregates are associated with replicative aging without compromising protein quality control. *Elife* 4.
- Schlissel G, Krzyzanowski MK, Caudron F, Barral Y, and Rine J (2017). Aggregation of the Whi3 protein, not loss of heterochromatin, causes sterility in old yeast cells. *Science* 355, 1184–1187. [PubMed: 28302853]
- Sharon E, Chen SA, Khosla NM, Smith JD, Pritchard JK, and Fraser HB (2018). Functional Genetic Variants Revealed by Massively Parallel Precise Genome Editing. *Cell* 175, 544–557 e516. [PubMed: 30245013]
- She R, Chakravarty AK, Layton CJ, Chircus LM, Andreasson JO, Damaraju N, McMahon PL, Buenrostro JD, Jarosz DF, and Greenleaf WJ (2017). Comprehensive and quantitative mapping of RNA-protein interactions across a transcribed eukaryotic genome. *Proc Natl Acad Sci U S A* 114, 3619–3624. [PubMed: 28325876]
- Shorter J, and Lindquist S (2004). Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. *Science* 304, 1793–1797. [PubMed: 15155912]
- Simchen G (2009). Commitment to meiosis: what determines the mode of division in budding yeast? *Bioessays* 31, 169–177. [PubMed: 19204989]
- Simons AM (2009). Fluctuating natural selection accounts for the evolution of diversification between hedging. *Proc Biol Sci* 276, 1987–1992. [PubMed: 19324774]
- Smits GJ, van den Ende H, and Klis FM (2001). Differential regulation of cell wall biogenesis during growth and development in yeast. *Microbiology* 147, 781–794. [PubMed: 11283274]
- Suzuki G, Shimazu N, and Tanaka M (2012). A yeast prion, Mod5, promotes acquired drug resistance and cell survival under environmental stress. *Science* 336, 355–359. [PubMed: 22517861]
- Tadros W, Goldman AL, Babak T, Menzies F, Vardy L, Orr-Weaver T, Hughes TR, Westwood JT, Smibert CA, and Lipshitz HD (2007). SMAUG is a major regulator of maternal mRNA destabilization in *Drosophila* and its translation is activated by the PAN GU kinase. *Dev Cell* 12, 143–155. [PubMed: 17199047]
- Tagkopoulos I, Liu YC, and Tavazoie S (2008). Predictive behavior within microbial genetic networks. *Science* 320, 1313–1317. [PubMed: 18467556]
- Tanaka M, Chien P, Naber N, Cooke R, and Weissman JS (2004). Conformational variations in an infectious protein determine prion strain differences. *Nature* 428, 323–328. [PubMed: 15029196]
- Tanaka M, Collins SR, Toyama BH, and Weissman JS (2006). The physical basis of how prion conformations determine strain phenotypes. *Nature* 442, 585–589. [PubMed: 16810177]
- Teste MA, Duquenne M, Francois JM, and Parrou JL (2009). Validation of reference genes for quantitative expression analysis by real-time RT-PCR in *Saccharomyces cerevisiae*. *BMC Mol Biol* 10, 99. [PubMed: 19874630]
- Toyama BH, Kelly MJ, Gross JD, and Weissman JS (2007). The structural basis of yeast prion strain variants. *Nature* 449, 233–237. [PubMed: 17767153]
- Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, and He C (2015). N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. *Cell* 161, 1388–1399. [PubMed: 26046440]
- Warringer J, Zorgo E, Cubillos FA, Zia A, Gjuvsland A, Simpson JT, Forsmark A, Durbin R, Omholt SW, Louis EJ, et al. (2011). Trait variation in yeast is defined by population history. *PLoS Genet* 7, e1002111. [PubMed: 21698134]
- Wickner RB (1994). [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* 264, 566–569. [PubMed: 7909170]
- Wickner RB, Edskes HK, Kryndushkin D, McGlinchey R, Bateman D, and Kelly A (2011). Prion diseases of yeast: amyloid structure and biology. *Semin Cell Dev Biol* 22, 469–475. [PubMed: 21345375]
- Wickner RB, Edskes HK, and Shewmaker F (2006). How to find a prion: [URE3], [PSI+] and [beta]. *Methods* 39, 3–8. [PubMed: 16793280]

- Winston F, Dollard C, and Ricupero-Hovasse SL (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* 11, 53–55. [PubMed: 7762301]
- Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, et al. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285, 901–906. [PubMed: 10436161]
- Wong BG, Mancuso CP, Kiriakov S, Bashor CJ, and Khalil AS (2018). Precise, automated control of conditions for high-throughput growth of yeast and bacteria with eVOLVER. *Nat Biotechnol* 36, 614–623. [PubMed: 29889214]
- Yofe I, Weill U, Meurer M, Chuartzman S, Zalckvar E, Goldman O, Ben-Dor S, Schutze C, Wiedemann N, Knop M, et al. (2016). One library to make them all: streamlining the creation of yeast libraries via a SWAp-Tag strategy. *Nature methods* 13, 371–378. [PubMed: 26928762]
- Zalatan JG, Coyle SM, Rajan S, Sidhu SS, and Lim WA (2012). Conformational control of the Ste5 scaffold protein insulates against MAP kinase misactivation. *Science* 337, 1218–1222. [PubMed: 22878499]
- Itakura & Chakravarty et al. demonstrate that we have been blind to the pervasive importance of protein-based inheritance in biology. Adaptive prediction of nutritional environments by [SMAUG +] prion polymorphs modulates cardinal growth and development strategies of *S. cerevisiae* both in the laboratory and the wild.
- Itakura AK, Chakravarty AK, Jakobson CM, and Jarosz DF (2019). Widespread Prion-Based Control of Growth and Differentiation Strategies in *Saccharomyces cerevisiae*.

- The [*SMAUG*⁺] prion allows yeast to anticipate nutrient repletion after starvation
- [*SMAUG*⁺] regulates the decision between cardinal growth and survival strategies
- [*SMAUG*⁺] is hidden in plain sight in common laboratory yeast strains
- Distinct [*SMAUG*⁺] variants are widespread in wild yeast populations

Figure 1. Tradeoffs between meiotic and mitotic proliferation in fluctuating environments

(A) (Left) Schema of the model framework. Mitotic proliferation is represented by logarithmic growth as a function of t_1 , the period of nutrient abundance. Meiotic proliferation is represented by a simple Hill function of t_2 , which captures differences in the rate of sporulation, but maintains the same maximum. (Right) Schema of the sporulator (S) and proliferator (P) developmental strategies. (B) (Left) Framework for altering the environmental parameters, duration of abundance and starvation, of the model. (Right) Example simulation showing the relationship between $\log_{10}(P/S)$ and nutrient starvation (t_2). Highlighted are the t_2 values where small changes would alter which developmental strategy is more successful. (C) Parameter space of simulated populations of proliferators relative to sporulators in fluctuating environments. For each plot, there are two independent variables: the period of starvation (t_2) and the title variable (changed at intervals; right bar). For all plots, the simulation was run for $N = 10$ cycles.

Figure 2. The laboratory strain BY4743 contains a prion

(A) Competitions between cured and uncured BY4743 were passaged every 48 hours (left) or every 24 hours (right). Each point represents the natural log of the number of cells expressing the mNeonGreen (Neon) fluorescent marker over the number of cells expressing the mKate2 (Kate) fluorescent marker (Wong et al., 2018; Zalatan et al., 2012), and the mean of three independent biological replicates. Filled dots represent data obtained when cured cells were marked with Neon and uncured cells were marked with mKate2. Unfilled dots represent the marker-swap experiment. Selection coefficients were calculated from a linear fit to these data ($p < 0.0001$ for all fitted lines; Chevin, 2011). Shaded area indicates the 95% confidence interval of the fitted line. Shown are the selection coefficients $\pm$ SEM of the reciprocal experiments. (B) (Top) Representative micrographs of cured and uncured BY4743 cells after 4 days of sporulation. White asterisks denote spores. Scale bar represents 10 μm . (Bottom) Sporulated fractions of cured and uncured BY4743 vs. time. Sporulation

rate (fraction sporulated/hour): BY4743: 6.9×10^{-4} ; cured BY4743: 1.2×10^{-3} . **: $p < 0.005$ by Mann–Whitney U test; n.s.: $p > 0.05$. (C) (Bottom) Sporulation efficiency of cytoductants with BY4741 and cured BY4741 as donor strains (p -value calculated by Mann–Whitney U test). (D) Sporulation efficiency of four sister progeny from diploid generated by crossing an uncured haploid crossed with a cured haploid P -values calculated by Sidak's multiple comparison test. A cured BY4743 is shown as a control. In all panels in this figure, error bars represent SEM from the mean of three independent biological replicates.

Figure 3. BY4743 harbors [SMAUG⁺]

Schema and sporulation efficiency of (A) the meiotic progeny of uncured haploid crossed to *vts1*⁻ and of (B) the *vts1*⁻ progeny from Figure 3A after the genetic restoration of *VTS1*. Bars represent means $\pm$ SEM from three independent biological replicates. (C) Growth rates of diploid and haploid cured, selectively cured, and uncured strains in low (0.08%) glucose. Means $\pm$ SEM from six independent biological replicates. (D) (Left) Schema of protein transformation. (Top) Growth rates of haploid protein transformants in low (0.08%) glucose. Bars represent means $\pm$ SEM from six independent biological replicates. (Bottom) Sporulation efficiency of selectively cured strains transformed with either Vts1 condensates or bovine serum albumin (BSA). Bars represent means $\pm$ SEM from four and ten independent biological replicates for the Vts1 and BSA transformants, respectively. All *p*-values in this figure were calculated by Mann-Whitney *U* test.

Figure 4. Natural [SMAUG⁺] hyperactivates Vts1

(A) Representative micrographs of BY4741, cured BY4741, and selectively cured BY4741 expressing either a galactose-inducible *GFP-SRE* (*SRE+*) or a permuted *GFP-SRE* (*sre-*) construct. DIC and GFP channels are shown. Scale bar represents 5 μ m. (B) Scatter plots of GFP fluorescence of BY4741, cured BY4741, and selectively cured BY4741 expressing either *SRE+* or *SRE-* constructs as indicated ($p < 0.005$ by Sidak's multiple comparison test). Bar denotes mean. N = 50–103 cells.

Figure 5. *MUM2* degradation suppresses sporulation in *[SMAUG⁺]* cells

(A) Schema of exit from mitosis into meiosis (Neiman, 2011; Simchen, 2009). (B) Ratio of induction of all transcripts induced during sporulation in *[smaug⁻]* cells vs. *[SMAUG⁺]* cells (in gray). Purple indicates a subset of the transcripts with annotated function in meiosis (SGD). P -value < 0.0001 by bootstrap t -test (C) The *[smaug⁻]/[SMAUG⁺]* ratio of induction of meiotic transcripts that were significantly induced in *[smaug⁻]* cells. (Inset) Schema of the *MUM2* transcript showing the location and sequence of the SRE hairpin loop. (D) Time course of *MUM2* expression during early meiosis in the uncured and cured BY4743, measured by qRT-PCR, normalized against *TAF10*. p -values were determined by Mann-Whitney U test. Bars represent means $\pm$ SEM from three independent biological replicates. (E) Sporulation of strains expressing *MUM2* under a strong promoter (*NOPI*; constitutive) and its native promoter (P -values were calculated by Mann-Whitney U test). Bars represent means $\pm$ SEM from three independent biological replicates. (F) Sporulation of cured and

uncured BY4743 with either a wild-type or permuted SRE found in the 5' - UTR of *MUM2*. P-values were determined by Mann-Whitney *U* test. Bars represent means $\pm$ SEM from three independent biological replicates.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 6. Natural *S. cerevisiae* strains harbor [SMAUG⁺]-like elements that impact sporulation (A) (Left) Heat map of the ratio of the mean GFP intensity in cured vs. uncured natural strains. BY4743 and [smaug⁻] are included as benchmarks. (Right) Violin plots of GFP fluorescence of cured and uncured SGRP strains expressing a *GFP-SRE* reporter for Vts1 activity. Dotted bars indicate upper and lower quartiles, and solid bars indicates medians. Data from strains with a significant increase in fluorescence upon chaperone curing are shown. *P*-values are determined by Mann-Whitney *U* test. N=106–936 cells. (B) Phylogenetic tree of the 21 successfully cured SGRP strains (Liti et al., 2009) denoting the effect of curing on GFP-SRE expression and sporulation.

Figure 7. Natural *S. cerevisiae* strains harbor distinct variants of [SMAUG⁺]

(A) Experimental schema. Lysates from natural *S. cerevisiae* were added to purified, fluorescently-labeled Vts1. After incubation, the samples were imaged to monitor condensate formation. (B) Representative images of fluorescently labeled Vts1 seeded with lysates from natural strains. A natural [smaug⁻] is included as a control. Scale bar is 5 μ m. (C) Condensate formation (quantified by average pixel intensity) vs. the strength of the [SMAUG⁺] activity (from Figure 6A). Blue dots indicate strains that harbor [SMAUG⁺]. Dashed line represents the linear regression line (slope = 0.01 ± 0.003 ; $p = 0.006$).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
KAPA SYBR Fast qPCR	kapabiosystems.com	Cat# KK4601
TURBO DNA-free™ Kit	Invitrogen	AM1907
Oligonucleotides		
Oligo dT-20 primer	Invitrogen	Cat# 8418-020
<i>HO</i> integration check reverse primer: GAG GGTATCTG G GCCTCCATG	This paper	N/A
<i>HO</i> integration 3' junction check forward primer: CCTGT AGAAGAGT A1 1 1 GTTGT	This paper	N/A
<i>HO</i> integration 3' junction check forward primer: GAAGCTAAGATTGGTAAGAAG	This paper	N/A
<i>Vis</i> forward primer: 1 1 1ATGCTAGACACTGCGGG	This paper	N/A
<i>Vis</i> reverse primer: AGCTGCTGTAGACGAAACTG	This paper	N/A
<i>TaFI0</i> forward primer: CGTGCACAGATT CACAAC 0	This paper	N/A
<i>TaFI0</i> reverse primer: CGACCTATATGAGCCCGTATTC	This paper	N/A
<i>MUM2</i> forward primer TGGATCCCGAGTCATGTTTG	This paper	N/A
<i>MUM2</i> reverse primer AAGCACCTGGGTC TGATTG	This paper	N/A
<i>SPS1</i> forward primer GATCGGCCATCGGCATATAA	This paper	N/A
<i>SPS1</i> reverse primer TGTGCTTAGTCTTGTGCAITCA	This paper	N/A
F primer for generation of pHJ2972 with <i>MUM2</i> gDNA TTATGATCCCAACATCTGTG MM	This paper	N/A
R primer for generation of pHJ2972 with <i>MUM2</i> gDNA ACCAAITGTTGGGATCATAGAATCA	This paper	N/A
Wild-type SRE homology template: GAAATGTAAACG ATAGTC AAACCTTAGTAAAGAGAATCAGCTGGCTTGGTACTTGTGATCTTTTGATTGACCTCATCTTTTGTGCAI ACACGGCTCGTTTGGAAATAC TGTGTGTA AAAATGAATTACATGGCTTATGACTACGACCGACCGCAGCATTCCTTGGAAAAAGTCTTTAAACAATTGGCATTCATCCCAACCAACAGTCACAG	This paper	N/A
Permitted SRE homology template: GAAATGTAAACG ATAGTCAAACTTAGTAAAGAGAATCAGTCCGCTTGGTACTTGTATC 777TGATTGACCTCATCTTTTGTGCAI ACACGGCTCG 777TGGAAATCTGTTGTAAA AATGAATTACATGGCTTATGACTACGACCGCCAGCATTCCTTGGAAAAAGTCTTTAAACAATTGGCATTCATCCCAACCAACAGTCACAG	This paper	N/A
Experimental Models: Organisms/Strains		
BY4741 <i>MA7a</i>	Winston et al., 1995	N/A
BY4742 <i>MA7a</i>	Winston et al., 1995	N/A
BY4742 <i>kar1-15</i>	Conde and Fink, 1976	N/A
BY4742 <i>vis1</i>	Giaever et al., 2002	N/A
Natural <i>S. cerevisiae</i> isolates	Lit et al., 2009	https://catalogue.neve.co.uk/sgrps
BY4743	Winston et al., 1995 Dharmacon.horizon-discovery.com	Catalog # YSCI050

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Recombinant DNA		
Hsp70 (K69M) plasmid	Jarosz et al., 2014a	N/A
Hsp70 (K69M) plasmid (Ura-selectable)	This paper	N/A
HO integration mKate2	Wong et al., 2018; Zalatan et al., 2012	N/A
HO integration mNeonGreen	Wong et al., 2018; Zalatan et al., 2012	N/A
Advanced Gateway destination vectors	Alberti et al., 2007	https://www.addgens.org/yeast-gateway/
pH2972 CRISPR/Cas9 construct	Anand, 2017	https://www.addgens.org/100956/
Software and Algorithms		
Matlab	MathWorks, Inc.	https://www.mathworks.com/products/matlab.html
ImageJ	NIH	https://imagej.nih.gov/ij/
Prism	GraphPad Software Inc.	https://www.graphpad.com/
Cell Profiler	Kamensky et al., 2011	https://cellprofiler.org/
FlowJo	FlowJo, LLC	https://www.flowjo.com/
DeSeq2	Love et al., 2014	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
Leica LAS X Core Image Analysis Software	Leica, Inc.	http://www.leica-microsystems.com/products/microscope-software/
Deposited data		
RNA sequencing	This paper	GSE138559
Mendeley Data	This paper	10.17632/kks382v4.1