Microbial specialization by prions

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ABSTRACT

Microbial prions facilitate a variety of phenotypic switches. Recently-developed tools that can directly interrogate, in the living cell, the aggregation state of a protein have enabled a wider range of experiments for prion-mediated behaviors. With such tools, the roles of the yeast prion [SWI+] in migration and mating were studied. Although [SWI+] cells were consistently less fit than their [swi−] counterparts under traditional laboratory conditions, in these new phenotypic paradigms [SWI+] cells demonstrated a distinct advantage. [SWI+] cells dispersed over a larger area under conditions resembling rainfall and outcrossed more frequently. We postulate that many behaviors in microorganisms may be modulated by stochastic prion switching. In diverse and changing natural environments, prion switching at low frequency may promote greater fitness of the population by specializing a small number of individuals with altered responses to their environments.

Functional prions

The three-dimensional conformation of a protein dictates its function in the cell. These states are not static. Often, proteins can perform multiple functions by adopting alternate conformations. An unusual class of proteins have the capacity to adopt a self-templating conformation, called a prion. These structures rapidly convert non-prion conformers of the protein into the prion conformer. This phenomenon forms the basis for some devastating neurodegenerative diseases in mammals, as aggregates of prion protein (PrP Sc ) readily incorporate soluble prion protein (PrP C ) into the aggregated state [1]. While these disease-causing prions were the first to be discovered, more and more functional prions, i.e. prions that serve a natural biological role, are being discovered in diverse organisms from bacteria to humans [2–7].

Yeast has been the archetypal model system for the discovery of prions [8]. Yeast have the opportunity to sample many different prion or non-prion conformations without high risk to the population on account of the relatively low frequency at which a cell gains or loses a prion conformation (~ 1 in 10,000,000 to ~ 1 in 100)[8]. Cells containing prions of diverse proteins grow faster than genetically-identical cells free of prions under certain environmental conditions [9–12]. This benefit propagates to future generations because prions are efficiently inherited by daughter cells through either mitosis or meiosis.

The first prion-forming proteins discovered in yeast harbored readily-distinguishable prion-forming domains [13–17], rich in glutamine and asparagine. By training computer algorithms to seek out other prion-like sequences rich in glutamine and asparagine, many domains capable of forming prions were discovered [18,19]. Such domains may also enable liquid-to-gel transitions, which alter protein function. Recently, the Sup35 prion-forming domain was shown to drive stress-induced, transient phase separation, improving fitness as cells recovered from stress [20]. Similar behavior has been demonstrated for a number of proteins harboring prion domains, suggesting that this phase transition capacity may be a typical property of the prion domain [21,22], potentially related to their ability to form amyloids. Thus, there may be a spectrum of protein activity changes mediated by self-assembly, where the prion state is the most stable of such assemblies. Moreover, if transient inactivation can provide a cellular benefit, then heritable inactivation may also be beneficial in particular environments, and vice versa. We anticipate the interrogation of additional prion-forming proteins and domains in the near future will demonstrate whether these properties are common features.

The once rapid pace of progress in identifying domains and proteins capable of forming prions has recently

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slowed considerably. The prions discovered in humans, including the first prion, PrP\textsuperscript{sc}, do not have distinguishable prion domains, nor do proteins with prion-like behavior identified in a recent unbiased screen in yeast \[12\]. With no apparent amino acid signatures among these newly discovered prions, we must return to empirical methods to identify new prions. Such a method should decouple the detection of prion states from their biological function, as it is challenging to predict \textit{a priori} what phenotype a prion might confer.

Recently, we reported the development of a new genetic tool to track protein aggregation that is especially suited for prions \[23\]. By fusing a protein of interest to a transcription factor that can drive GFP expression, yTRAP (yeast Transcriptional Reporting of Aggregating Proteins) connects the solubility state of that protein to GFP fluorescence. Thus, a transition from a soluble state to an insoluble state produces a sharp reduction in GFP production and with it a loss of fluorescence. With this tool, conformational changes to a prion state can be detected by a long-lived, heritable change in fluorescence following a short perturbation, such as transient overexpression or a brief stress. While the absolute level of the yTRAP signal can vary depending on the protein of interest, there should be no difference between the pre-perturbation and post-perturbation signals for any protein unless it undergoes a sustained structural transition, such as a prion switch. In addition to providing the capacity for discovering new prions, yTRAP simplifies a wide range of experiments with prion-containing strains because it enables tracking prion states at the single cell, colony, or population level. By enabling facile differentiation between cells that contain the prion and those that do not, it allows for the tracking of prion state during complex microbial behaviors.

The \([SWI^+]\) prion specializes ‘settler’ cells into ‘pioneers’

The Swi1 protein is a yeast chromatin remodeling factor that can adopt a prion conformation, \([SWI^+]\) \[24\]. As in other examples of yeast prions, the prion state \([SWI^+]\) confers a partial loss of function of the Swi1 protein \[24–27\]. However, no common environmental condition has previously been demonstrated to confer a robust growth advantage for the \([SWI^+]\) prion state. Some downstream consequences of Swi1 loss-of-function and \([SWI^+]\) prion formation are known. These include downregulation of the homothallic gene \(HO\) \[28\], responsible for mating-type switching, and the downregulation of \(FLO\) genes, leading to a loss in both flocculation and substrate invasion \[29\]. The latter consequence was hypothesized to enhance the migration of \([SWI^+]\) cells \[29\]. We set out to develop new experimental procedures to mimic environmental situations where these phenotypes could be relevant. Instead of studying growth in isolated cultures, we employed the unique functionality of yTRAP to study the influence of the \([SWI^+]\) prion on cell dispersal and outcrossing behaviors in yeast.

In order to grow at the maximal rate under ever-changing conditions, microbes employ a broad range of behaviors. Even without a discernable improvement of growth rate, altering behavior can result in new opportunities for microbes and help to produce larger overall populations. At times, it may be beneficial to stick together and form biofilms that resist assault, while at other times it may be beneficial to spread out over a larger area. Despite the slower growth of \([SWI^+]\) cells, they were less adherent, meaning they could more readily disperse in flowing water. When we grew the cells in conditions resembling rainfall, those harboring \([SWI^+]\) colonized a
larger swathe of nutrient sources and grew to a larger biomass than their genetically-identical [swi−] counterparts (Figure 1) [30]. Clearly, the enhanced ability of [SWI+] cells to explore and colonize new territory would be, under many circumstances, an advantageous trait.

Next, we examined what advantage might be conferred by the changes in yeast mating behavior induced by the [SWI+] prion. With a functional HO gene, most haploid S. cerevisiae cells divide once, after which mother cells switch mating types and mate with their daughters [31]. While this returns them to the more robust diploid state, it also results in complete homogyosity. Cells with the [SWI+] prion suppress the expression of HO and are less likely to switch mating types. Therefore, they are more likely to mate with unrelated, genetically diverse partners [30]. This behavior might be considered more risky, since it could result in the acquisition of new prions, transposons, or undesirable mutations from the mating partner. However, the greater diversity of genetic material that [SWI+] cells would likely acquire also has the potential to confer beneficial traits.

We examined only two microbial behaviors distinct from growth rate, both of which we intuitively predicted would be affected by [SWI+] based on existing knowledge. [SWI+] had potential advantages in both behavioral paradigms. On account of their increased propensity to explore more genetic and geographic space, we termed [SWI+] cells ‘pioneers,’ and the robust and stationary [swi−] cells ‘settlers.’ Swi1 is a pleiotrophic chromatin remodeler and its prion form may confer many other new phenotypes besides those currently examined in the laboratory. Other prions could also impact these phenotypes and behaviors. In S. cerevisiae, many different behaviors are regulated by the FLO11 gene including surface adhesion, colony morphology, pseudohyphal growth, and invasion. The [MOT3+] prion has been shown to regulate FLO11 and can modulate most of these phenotypes [32]. In addition, at least two other yeast prion proteins can regulate FLO11 transcription – [URE3] and [CYC8−] [32]. Each one could affect FLO11-dependent phenotypes in combination with unique sets of other genes regulated by each factor.

Another hallmark behavior of S. cerevisiae is ‘glucose repression.’ In the presence of glucose, even in small amounts relative to other carbon sources, S. cerevisiae will shut off other catabolic pathways to focus exclusively on utilizing the glucose. This stereotypical programming can be overcome by presence of the [GAR+] prion, which causes cells to lose glucose repression and become metabolic generalists [11,33,34]. We propose that the ability to alter or inactivate common behaviors using reversible prion switches provides a means to adapt to circumstances in which those behaviors are disadvantageous. Because of the complex and changing environment, such circumstances may arise quite frequently on an evolutionary timescale. Losing typical behavior through prion switching, as opposed to mutation, permits cells to readily re-acquire the old adaptive behavior once the environment changes again [35].

Outlook on prions in specializing microbial behaviors

Prions are not restricted to S. cerevisiae. They also exist in the distantly related fungus Podospora, where [Het-s] regulates heterokaryon incompatibility [36] and is thought to protect against viral infection. The bacterium E. coli was recently shown to propagate prions, including a prion form of Rho from Clostridium botulinum [7,37]. In bacteria, many behaviors have been characterized, any or all of which may be modulated at some frequency by prion switches. Akin to the behaviors regulated by [SWI+], bacteria also exchange genetic material through conjugation and express adhesins to attach to other cells and substrates. Many bacteria have active motility mechanisms that respond to light, heat, and nutrients. Bacteria communicate with each other through quorum sensing and can generate or dissolve biofilms. Some bacteria switch at low frequencies into a dormant persistor state, which is thought to be a bet-hedging mechanism allowing those cells to survive rare but otherwise deadly antibiotic treatment. Could they also use prion switching to hedge their bets against predictable and sometimes detrimental behaviors?

Prion states that modulate behaviors could benefit microbial populations by specializing subpopulations to particular ecological niches or purposes (Figure 2). [SWI+] pioneer subpopulations in S. cerevisiae could allow the species to colonize more territory and mate to acquire beneficial genetic material without excessive risk to the population. We speculate that this phenomenon of prion-based specialization may also be present in other eukaryotic microbes and bacteria. For example, prions that cause bacteria to shun biofilm formation could enhance their dispersal and migration, or prions that halt sending/receiving quorum sensing signals may allow this subpopulation to evade predators or host immune responses.

Recently-developed techniques will enhance exploration into prion proteins and the behaviors they regulate. Distributed Amphifluoric Förster Resonance Energy Transfer (DAmFRET), a technique using a photoswitchable molecule that can undergo FRET with its non-converted self, is well-suited to the discovery of new prions. Using DAmFRET, the ability of a protein to exist in multiple conformations manifests as an increase in FRET at higher protein expression levels [38]. yTRAP can also be used to screen sets of proteins for the ability...
to switch to a prion state and is able to report on the prion state of single cells or colonies using fluorescent photogra-phy [23]. This ability was useful in the study of [SWT]-dependent behavior and could be used to track prion states through other behavioral studies. Because the output of yTRAP is programmable, it can report in a number of modalities and can even be used to engineer cellular devices out of prion switches [23]. These techniques report directly on the conformational state of prote-in-of-interest and therefore are poised to the exploration of new and diverse prions. DAmFRET and yTRAP should be ported to other organisms to enable the investigation of yet more novel prions and phenotypes.

Prion-switching in fungi and bacteria allows genetically-identical cells to diversify their pre-programmed behavior. Prion domains confer the ability for a protein to be rapidly modulated post-translationally, without the need to evolve specific transcriptional regulation over it. Moreover, the formation of prion strains, prions of varying phenotypic strength, allows the exploration of a range of protein activity levels in parallel [8]. Surviving cells with beneficial prion states faithfully pass these prions on to progeny. Changing environments may select for new prion states, but the potential to return back to the original prion state when the environment reverts is always present. Bacteria and other microbial species are often thought to reproduce clonally. However, the ability of prions to regulate growth and behavior offers the opportunity for even genetically-identical cells to specialize in unique ecological roles.

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Disclosure statement

No potential conflict of interest was reported by the authors.
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