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Blessings in Disguise: Biological Benefits of Prion-Like Mechanisms

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Abstract

Prions and amyloids are often associated with disease, but in fact, related mechanisms provide beneficial functions in nature. Prion-like mechanisms (PriLiMs) are found from bacteria to humans where they alter the biological and physical properties of prion-like proteins. We have proposed that prions can serve as heritable bet-hedging devices for diversifying microbial phenotypes. Other, more dynamic, proteinaceous complexes may be governed by similar self-templating conformational switches. Additional PriLiMs continue to be identified and many share features of self-templating protein structure (including amyloids) and dependence on chaperone proteins. Here we discuss several PriLiMs and their functions, intending to spur discussion and collaboration on the subject of beneficial prion-like behaviors.

Keywords: Prion, amyloid, prion-like, PriLiM, bet-hedging, RNP granule

Glossary Boxes (Editor: For display in side bar. The first occurrences are bolded in the main text):

Amyloid-like (amə¦loid): This term is used loosely here and in the literature to describe species that 1) might be true amyloids but are not yet fully characterized (i.e. not yet known to have cross-beta structure), or 2) share some amyloid characteristics but definitely not all (i.e. forming self-templating fibers but not SDS-resistant or Thioflavin-T binding).

PriLiM (prē' lim): Prion-like mechanism. A phenomenon involving the propagation of a self-templating switch in protein conformation.

PriLiP (prē' lip): Prion-like protein. Any protein that can propagate its conformation via a prion-like mechanism (PriLiM).

Defining prions, amyloids, and similar phenomena

Prions have been defined as "infectious proteins" that can assume a profoundly altered conformation and propagate that conformation in a self-templating process. The mammalian prion protein, PrP, is the founding example of such self-propagating conformations and it is the only established prion that is infectious to humans. The best characterized prion proteins are found in fungi, where their self-propagating states are transmitted to mating partners and progeny as epigenetic elements of inheritance. A highly sophisticated system of remodeling factors ensures that the prion template is divided into oligomeric prion seeds that are inherited with very high fidelity [1]. Most of these prions form an unusually stable aggregate structure known as an amyloid fiber, which is typically defined by three characteristics: (1) a structure consisting of beta strands running perpendicular to the axis of the fiber, resulting in a stereotypical cross-beta diffraction pattern (2) high stability characterized by resistance to heat and SDS denaturation, and (3) binding to hydrophobic dyes such as thioflavin T and Congo Red. Owing to their unique physical properties, nature has also made extensive and diverse use of amyloids ranging from bacterial biofilm components to melanin scaffolding in humans [2–5].

However, not all related phenomena fit squarely into the categories of prions and amyloids. Several mechanisms have been described as "prion-like," meaning that an initial

conformational change of a protein can template the conversion of other proteins to a similar or identical conformation [6–8]. Unlike *bona fide* prions, these need not be transmissible between individuals. Some mechanisms have been called "**amyloid-like**," meaning that they have some but not all of the properties of amyloids [9–11]. Amyloid and amyloid-like aggregates are subsets of prion-like phenomena because they template soluble proteins to adopt their fold as proteins are added to the aggregate. In this opinion, we illustrate the breadth of beneficial prion-like mechanisms (**PriLiMs**) and their cognate prion-like proteins (**PriLiPs**) by discussing several examples of the functions they provide: building stable structures, signal propagation, dynamic scaffolding of ribonucleoprotein (RNP) granules, and bet-hedging in microorganisms.

Bet-hedging prions enhance phenotypic diversity and adaptation in microorganisms

Bet-hedging mechanisms are used to diversify microbial phenotypes. In fluctuating environments this allows some fraction of the population to 'win' and thrive in conditions when most would 'lose,' or perish [12], [13]. For example, bacterial persister cells can survive antibiotic treatment, potentially saving the population of bacteria from extinction. The cost of this mechanism is that, until they switch out of their persistence phenotype, such cells grow much slower than normal cells in the absence of antibiotics. Even though antibiotics may be encountered rarely and persister cells have a severe growth defect, it is advantageous for the species to conserve this bet-hedging mechanism to survive occasional exposure to such strenuous environments [14].

Similarly, we've found that fungal prions produce a variety of new phenotypes that are often disadvantageous but can provide great advantages in particular circumstances [15–19]. We've proposed that such prions act as bet-hedging mechanisms: at a low frequency in a population of yeast cells, prions conformations are nucleated, resulting in a heritable, altered activity that underlies a phenotypic change. Due to the self-propagating nature of prions and to the mechanisms that ensure their orderly distribution to progeny, prion phenotypes are heritable. Rare cells, however, switch back to the non-prion state when they lose the prion template. A recent example of a prion that confers antibiotic resistance is the yeast prion [*MOD*$^{+}$] (nomenclature of yeast prions: see Box 1) [20], [21]. [*MOD*$^{+}$] cells are resistant to azole-based antifungals, but in rich media they have a growth disadvantage. To date, bet-hedging functions for prions have only been described in *Saccharomyces cerevisiae.* However, many findings suggest they are widespread through the microbial world (see below and Box 1) and we expect that more will soon be discovered elsewhere.

Three independent studies predicted prion-like sequences in the *S. cerevisiae* genome computationally [22–24], one following up with experimental evidence of prion-like behavior [23]. Each study identified sets of proteins that were significantly enriched for regulatory functions – transcription factors and RNA-binding proteins. Importantly, because these proteins regulate many genes that often act cooperatively, bet-hedging prions involving such factors could allow cells to immediately acquire complex, heritable phenotypes [15–17]. Some prion states may confer "pre-adapted" complex phenotypes to enhance survival in environments that are encountered rarely but repeatedly, for which bet-hedging strategies are favored [12], [25]. Other prions may act as evolutionary capacitors allowing random variation

to accumulate cryptically for many generations before being tested by a small proportion of the population [26]. An example of this is the prion [*PSI*$^{+}$], formed by a translation termination factor. The [*PSI*$^{+}$] prion allows ribosomes to read through stop codons, uncovering previously-silent genetic variation on a genome-wide level [27]. Phenotypes that provide a consistent advantage can become "fixed" in the genome, that is, independent of the prion, by the accumulation of new mutations or by the genetic reassortment of pre-existing variation [18], [19].

The phenotypes produced by conformational changes in a prion protein can be compared to the phenotypes that are created by genetic mutations [28], [29]. In many cases, the prion conformations are self-propagating amyloid states that are inactive, similar to loss-of-function or null mutations in genes. Furthermore, most prions can adopt multiple amyloid conformations with different fragmentation and elongation rates. These create prion 'strains,' that have unique ratios of soluble:amyloid protein and thus different activity levels [30]. These prion strains are akin to an allelic series of a gene, tuning the level of a protein's activity, and thus the phenotypic consequences of the prion state [31].

Depending on the genetic background and the particular prion protein involved, *S. cerevisiae* prion proteins switch between prion and non-prion states at frequencies between 10^{-2} and 10^{-7} [31–34]. Thus, prion-based phenotypes can be sampled much more frequently on average than loss-of-function mutations. Furthermore, because prion inheritance depends on protein homeostasis machinery (see Box 1), they might quite naturally switch more frequently under conditions that stress protein homeostasis, that is, when cells aren't well-adapted to

123 their environment (see Fig. 1). This has in fact been observed for [*PSI*$^{+}$] [32], [35]. Increased
124 switching under stress could be of great advantage to a population of yeast, allowing
125 individuals to sample multiple, potentially life-saving phenotypes when they most need
126 them. This, in effect, changes the bets that the population of yeast has on the table. If the
127 stress persists, those few cells that survive pass on to their progeny the protein state(s) that
128 saved them.

129 There are also cases where specific stresses induce specific prions. This is likely to occur
130 for more predictable conditions that cells encounter regularly, allowing them to evolve a prion
131 response which increases viability in the new environment (see Fig. 1). Ethanol was observed
132 to increase the appearance of the yeast prion [*MOT3+*] (Halfmann and Lindquist, unpublished
133 data), which de-represses anaerobic genes, while certain bacterial competitors could induce the
134 yeast prion [*GAR+*] (Jarosz and Lindquist, unpublished data), which overcomes glucose
135 repression. In both cases, [*PSI+*] is not induced, so prion induction appears specific, however
136 the mechanisms of induction remain unknown.

137 Environmental adaptation via bet-hedging prions has two major advantages over
138 adaptation through genetic mutation: (1) it allows a microbial population to have diverse,
139 heritable, and complex responses to environmental conditions, even when the population is
140 not large enough for substantial genetic diversity, and (2) bet-hedging prions allow for fast
141 reversion from a loss-of-function or “null” prion state of a protein, when reversion from a loss-
142 of-function mutation at the DNA level is quite rare.

To exemplify the first point, a small yeast colony growing on a plant may gain benefit from having some members stay attached while others detach to follow the flow of rainwater and spread the population. Prions that regulate surface adhesion may be ideal to promote colony diversity. Indeed, a wild strain of yeast was recently found to adhere to agar growth medium after washing only when the translation termination factor Sup35 was in its prion conformation, the [*PSI*$^{+}$] state [19]. Additionally, the *FLO11* gene in yeast, which is a central regulator of colony morphology and adhesion, is regulated by multiple well-characterized yeast prions – *URE2, CYC8*/*SSN6, MOT3, SFP1*, and *SWI1* all affect its transcription [36–42]. Besides adhesion, prions confer a number of different phenotypes, which vary from strain to strain, that could be used to diversify small populations. Consistent with this, the growth of [*PSI*$^{+}$] and [*psi*$^{-}$] yeast have been compared across many conditions, and quite often one state or the other confers a marked benefit to growth. [17], [18], [35].

The second advantage of bet-hedging prions, the relatively fast rate of reversion from a hypomorphic change in activity due to the prion state, derives from the frequency at which loss-of-function mutations are beneficial to organisms. By far, the most common genetic mutations sampled are loss-of-function, and frequently these are adaptive. It may be beneficial to lose the function of a gene because of the energy cost associated with it or because new environmental conditions disfavor the original gene [43–45]. However, microbial populations cannot adapt exclusively to their current environment at the expense of all others, because conditions in nature are always in flux (see Fig. 2). Summer and winter, dry and wet, nutrient-rich and nutrient-poor conditions, are just a few examples of the cycles that many organisms must have adapted to in order to have survived to the present. At the same time, new

environments are also being sampled with different intrinsic physical properties and changing microbial competitors. *S. cerevisiae* was recently shown to undergo such drastic environment changes as to live on grapes in the summer and to survive the winter in the gut of wasps [46]. A null mutation that is favorable in one environment could easily be deleterious in the next set of environments, which will consist of both familiar and novel elements, but genetic changes revert at a rather low frequency. Bet-hedging prions allow organisms to rapidly acquire and revert from loss-of-function phenotypes and other sampled traits, testing new phenotypes and resampling expression programs that were advantageous in the past (see Fig. 2).

While bet-hedging prions have so far only been observed in fungi, we expect that more will soon be discovered in other microbes. The first yeast prions identified in *S. cerevisiae*, Sup35 and Ure2, have domains rich in glutamine and asparagine residues (Q/N-rich or "prion-like" domains). This unusual feature was successfully used to identify other *S. cerevisiae* proteins that could behave as prions and modulate the activity of a fused reporter [23] (22 of the 90 tested Q/N-rich proteins could do this, or 24%). To our knowledge, no screen has been conducted to search for prion-like domains in the abundance of protozoan genomes that have recently become available. In 2000, Michelitsch and Weissman surveyed the 28 prokaryotic genomes that were available at the time, but found few Q/N-rich sequences compared to the content of *S. cerevisiae* [22]. On the other hand, an enormous 24% of proteins in *Plasmodium falciparum*, the causative protozoan parasite of malaria, are Q/N-rich [47], compared to 1.5% of *S. cerevisiae* proteins, and 0.3% of human proteins [22], [48]. Furthermore, a computational analysis found that the propensity to form amyloids increases as organism complexity decreases [49], but the only single-celled organisms screened were *S. cerevisiae* and

187 *Paramecium tetraurelia*, both eukaryotes. Clearly, a high-throughput analysis of the thousands
188 of microbial genomes available could provide a wealth of information regarding potential bet-
189 hedging prions.

190 It is important to note, however, that not all yeast prions contain Q/N-rich sequences.
191 The Het-s prion of the fungus *Podospora anserina* [50] and the *S. cerevisiae* prion Mod5 [20] are
192 both able to form amyloids and propagate heritably even though they lack any Q/N-rich
193 domain. Furthermore, some yeast prions do not form amyloids at all – the prion [GAR^{+}]
194 appears to consist of a self-propagating, non-amyloid interaction between two proteins, the
195 proton pump Pma1 and the glucose signaling protein Std1 [51]. Another prion, [β], consists of a
196 self-activating vacuolar protease [52].

197 The evolutionary benefits of bet-hedging prions are just beginning to be explored and
198 remain controversial. An alternative hypothesis is that the ability of many prions to form
199 amyloids is an undesirable disease state [53]. Indeed, for essential yeast prion proteins like
200 Sup35, some amyloid strains that have been generated by overexpression are so strong that
201 they deplete cells of its essential activity, which kills them [54]. However, even if this lethality
202 occurs at natural expression levels, it could be an acceptable cost for the benefit of adaptability
203 that bet-hedging prions provide to the population [15], [16]. Throughout evolution,
204 detrimental mutations are experienced much more frequently than beneficial ones, yet
205 mutations remain the dominant force in evolution. It is difficult to assess the impact of prion
206 switching over the course of evolutionary history because no direct trace is left
207 behind. However, comparative genomics may be one method of determining how some prions

have been utilized in the past [55]. Others include determining the conservation of prion-forming domains and examining snapshots of adapting cells recently taken from their natural habitat. A recent study surveying 700 wild *S. cerevisiae* isolates found that prions were present in at least one third of the strains [19]. Prion loss was induced by transiently inhibiting a chaperone involved in maintaining prions. When assayed in 12 different growth conditions, prion loss frequently conferred a growth disadvantage. Thus, these prions had adaptive value. It is likely that these results underestimate the number of cells that are utilizing prions in natural populations because only a small number of conditions were tested.

Further supporting the usefulness of prions in fungi, Medina and colleagues observed broad conservation of many prion-like domains [56]. The authors computationally searched through the 103 sequenced fungal genomes for homologs of 29 Q/N-rich proteins that can function as prions in *S. cerevisiae* [23]. Strikingly, >99% of the fungi have at least a few homologous proteins containing Q/N-rich domains – only one distant relative lacked any such homolog. It remains to be shown whether these fungal prion-like domains function as bet-hedging prions, or as another kind of prion, or whether their behavior is not prion-like at all. However, several of the Sup35 homologs were able to propagate the [*PSI*$^{+}$] prion in *S. cerevisiae* [57–59]. It seems likely that prions are widely used as bet-hedging devices throughout fungi and in other branches of life as well.

Bet-hedging strategies like this may or may not be employed by more complex, multicellular organisms. These provide a specialized and more stable environment (or niche)

for most cells and typically produce fewer progeny. Nevertheless, many other uses for PriLiMs have been identified, several of which we will discuss below.

Amyloid-based PriLiMs have useful physical properties

Some prion-like mechanisms (PriLiMs) composed of self-templating amyloids are highly regulated and are activated reliably in response to particular signals. These functional protein complexes do not act as genetic elements. Some are used for the physical properties that an amyloid fiber provides, scaffolding meshworks, coating surfaces, or binding to pigments. These phenomena have been well-reviewed elsewhere as types of functional amyloids [2–5], and we will only briefly mention their functions.

In microorganisms, the physical properties of extracellular amyloids have been used to alter cellular interactions with surfaces. Diverse bacteria use amyloid fibers as a component of biofilms, which help to accumulate nutrients and protect bacteria from harsh conditions [60], [61]. It was recently proposed that cell surface proteins in yeast also mediate biofilm attachment and function as amyloids [62]. Both bacteria and fungi are able to coat themselves with amyloid fibers made of proteins called chaplins and class I hydrophobins, respectively [63]. These proteins can enhance attachment of the microbe to a host, or allow it to escape an aqueous environment and spread spores through the air.

PriLiMs used for their physical properties are also found in metazoa. Insects and fish use amyloid fibers as eggshell components [2]. In humans, Pmel17 forms amyloid fibers that bind toxic melanin precursors and scaffold their polymerization in melanosomes, which are subsequently transferred to surrounding cells [64]. Recently, a variety of hormone peptides

were found to be stored in an amyloid state in mammalian pituitary secretory granules [65]. The widespread use of these PriLiMs establishes amyloid formation as a common structural state that, when adopted, alters the physical properties of proteins.

Stable PriLiMs as a part of biological signaling cascades

Prion-like aggregation can also alter biological activity, changing interactions with other macromolecules. Several phenomena have recently been described in which prion-like aggregation is used to propagate a biological signal, providing a gain-of-function for the constituent protein or proteins (see Fig. 3).

Two such PriLiMs are involved in antiviral signaling. The first mechanism involves a templated conformational change of the human mitochondrial anti-viral signaling (MAVS) protein on the surface of mitochondria to a fibrous state [6]. The initial conformational switch appears to be templated by the RIG-I protein when it binds to double-stranded viral RNA in the cytoplasm. In its assembled form, MAVS interacts with TNF receptor associated factors (TRAFs) and propagates a signal that results in the induction of type I interferons and other antiviral molecules [6]. The second mechanism can be triggered by *Vaccinia* virus, which inhibits caspases to prevent the host cell from undergoing apoptosis [66–68]. When this happens, another cellular death mechanism is deployed. The cellular kinases RIP1 and RIP3 interact and rapidly form amyloid fibers [67]. In the amyloid state, the kinase domains of RIP1/3 are activated and phosphorylate downstream targets to cause programmed necrosis of the cell and an inflammatory response in the surrounding tissue [67], [69]. Such signaling PriLiMs may be used at key steps in antiviral responses because viruses might have more difficulty evolving

mechanisms to interfere with self-templating amyloid assembly than with signaling cascades which are inherently reversible. Such mechanisms are not likely to be restricted to mammals.

Another signaling PriLiM is the self-perpetuating conformation of cytoplasmic polyadenylation element binding protein (CPEB) from the neurons of the sea slug *Aplysia*. In its non-prion state, CPEB binds and inhibits the translation of mRNAs that are involved in building stable synapses [70]. The repeated stimulation of neurons with the learning-associated neurotransmitter serotonin causes the assembly of CPEB into an amyloid state. CPEB gains activity in this form, enhancing the translation of target mRNAs. This plays a major role in strengthening and stabilizing synaptic boutons for long-term potentiation [71], [72]. The *Drosophila* homolog Orb2A also forms oligomers in neurons that are required for the stabilization of long-term, but not short-term memory. The removal of the prion-like domain in Orb2A abolishes long-term memory. Mammals also express several CPEB proteins that contain Q-rich domains in neurons, but whether prion conversion contributes to memory in mammals is not yet established [73]. Certainly, a self-perpetuating PriLiM such as CPEB seems an ideal way to perpetuate the memory of stimulation for long periods of time, with the large size of the complex keeping it local and synapse-specific.

Astonishingly, when neuronal *Aplysia* CPEB was expressed in yeast, it readily assembled into a heritable, prion-like state [7], [74]. The activity of the CPEB increased in this prion-like state, as it does in neurons, activating the translation of target mRNAs containing its recognition sequence – a cytoplasmic polyadenylation element. This demonstrates that stable PriLiPs from other organisms, even ones that are only present in differentiated, non-dividing

cells, can be propagated indefinitely as prions in yeast. Using yeast as a model for these mechanisms could be of great advantage for studying phenomena from less genetically-tractable organisms.

Like *Aplysia* CPEB, some endogenous yeast prions may have altered function, rather than simply decreased function, in the prion state. The [*ISP*$^{+}$] prion does not confer the same phenotypes found in a *Δsfp1* strains, but rather the additional phenotype of nonsense antisuppression [75].

It is unlikely that stable PriLiMs are used exclusively for either their physical properties or for signaling, but rather for a combination of both. An interesting avenue for future research is to determine how the physical structure of amyloids may help to scaffold the interactions of signaling PriLiPs, and how amyloids that are used for their physical properties, such as CsgA in biofilms, may alter their interactions with binding partners upon assembly.

Dynamic PriLiMs help to form reversible RNP granules

Prion-like domains are also involved in the assembly of dynamic ribonucleoprotein (RNP) granules that process and modify RNA. While it has been known for some time that Q/N-rich, Q-rich, or other low complexity domains are essential for forming some RNP granules [8], [76], [77], how these large assemblies are regulated and structured remains elusive. Unlike amyloids, stress granules are composed of many different proteins which can undergo rapid exchange with the cytoplasm [76], [78]. Recently, a clue to this puzzle was found by Han, Kato, and colleagues. Even with no RNA present, many RNPs could be precipitated together from mammalian cell extracts using a crystalline compound that is thought to mimic the surface of a

cross-beta sheet [9], [79]. The retention of GFP-tagged protein in a hydrogel composed of the RNA-binding protein FUS provided an *in vitro* assay for interactions between these low-complexity sequences. The FUS fibers comprising the hydrogels were amyloid-like as assessed by their stereotypical diffraction pattern and appearance by electron microscopy. But unlike amyloids, these assemblies could incorporate different proteins, were rapidly reversible, and were not SDS-resistant. Thus, concerted, templated conformational changes among different low-complexity domains could be the basis of RNP granule formation.

Such a mechanism is prion-like in that one protein templates another to fold into the same basic structure, but is different from other PriLiMs because it is much more dynamic, perhaps allowing the segregation of interacting domains into a 'liquid' or gel-like phase separated from the rest of the cytosol [80], [81]. Phosphorylated FUS monomers no longer interact with the assembled FUS hydrogel, suggesting that assembly could be regulated by post-translational modification [79].

A screen of Q/N-rich domains in yeast identified several RNP granule components with domains that could act as yeast prions, and perhaps have bet-hedging functions [23], [82]. Nrp1, Pub1, and Hrp1, which associate with yeast stress granules, and Lsm4, which contributes to P body formation, could all form amyloid fibers and propagate the activity state of a fused reporter [23]. Notably, like FUS fibers, Hrp1 fibers were not SDS-resistant. The physical state of these yeast proteins in such RNP granules remains to be determined, but they may well assemble in a dynamic fashion. If, instead of forming such reversible assemblies, a small

fraction of the population inactivates these RNA binding proteins by nucleating an amyloid, it might serve as a bet-hedging mechanism to diversify cellular phenotypes

RNP granules are found broadly throughout eukaryotes – some regulate RNAs spatiotemporally in gametes and embryos, while others are used to transport RNA down neuronal dendrites [78]. How these dynamic complexes are assembled and regulated *in vivo* at a molecular level is still largely unknown, and will be a fascinating avenue of future research.

Concluding remarks

We have discussed several biological functions that prion-like mechanisms (PriLiMs) have in nature. It is likely that many more PriLiMs await discovery in diverse cellular pathways. In *C. elegans*, 1% of proteins have Q/N-rich, prion-like domains, and in *Drosophila* the fraction is even greater, 3.5% [22]. Some might function as stable or dynamic PriLiMs, and a small number may even have bet-hedging functions. The yeast prions [GAR^+], [Het-s], and [MOD^+] demonstrate that even proteins without canonical prion-like domains can function as prions. The real number of self-templating PriLiMs functioning in nature may be much greater than we can currently predict by sequence.

Despite the diversity of PriLiMs, some basic principles are likely to be shared. For example, they may all take advantage of the cells core protein homeostasis machinery. The *S. cerevisiae* prion proteins investigated to date all depend on Hsp104 and/or Hsp70 [20], [23], [48], [51], [83]. MAVS aggregation in extracts from human cells appears to be dependent on Hsp90 [6], and mammalian stress granule regulation involves Hsp70 and perhaps other chaperones [8]. *Aplysia* CPEB is readily propagated in yeast where it forms a yeast prion, and is

353 also subject to chaperone activity [7]. These connections to protein homeostasis may make
354 them intrinsically responsive to diverse internal and extracellular conditions. This, however, is
355 clear: prion-like mechanisms are not restricted to disease, but are broadly used for the benefit
356 of life.

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Box 1: Yeast prions confer non-Mendelian traits and depend on chaperones to propagate

In 1994, prion propagation was proposed to explain some perplexing, non-Mendelian phenotypes identified in yeast [84]. A yeast prion segregates in a non-Mendelian fashion because it is not based on a mutation in DNA inherited through chromosomes, but rather on a self-propagating protein conformation inherited through the cytosol. If a cell containing the prion state of a protein (a [*PRION*$^{+}$] cell) mates with a cell containing that protein in a nonprion state (a [*prion*$^{-}$] cell), the nonprion proteins are rapidly templated and take on the self-propagating prion conformation. Because all meiotic progeny inherit part of the parental cytosol, the vast majority will display the prion phenotype, rather than 50%, as one might have expected if the phenotypes were based on two different alleles of a gene. We refer the interested reader to these excellent reviews on yeast prion biology [31], [85], [86].

The [*PRION*$^{+}$] / [*prion*$^{-}$] nomenclature is used for all yeast prions - square brackets indicate the non-Mendelian segregation of the prion phenotype; capital letters indicate the dominant phenotype in mating (the self-propagating conformational change), while lower-case letters designate the recessive phenotype usually associated with soluble, un-templated protein.

Chaperones are intimately involved in prion propagation – perturbing chaperone function often results in an increased rate of prion appearance or loss (or both) [31], [35], [87]. The majority of fungal prions rely on Hsp104 [20], [23], a protein disaggregase that can sever amyloid fibers and generate new ends for growth [87]. By inhibiting this enzyme over several generations, [*prion*$^{-}$] cells can be reliably generated from a [*PRION*$^{+}$] population [88]. Hsp104

cooperates with Hsp70 and Hsp40 to exert this prion-propagating activity in a delicately balanced process that seems to have been fine-tuned to allow for prion propagation [87]. One prion, [*GAR*$^{+}$], does not appear to result from an amyloid conformation and is not dependent on Hsp104, but still requires Hsp70 to propagate into daughter cells [51].

Homologues of all of these chaperones are found broadly throughout many branches of life, perhaps indicating a conserved ability to propagate prions. Bacterial homologs were recently found to be capable of replacing yeast chaperones to propagate a prion in yeast [89], and yeast prions have been successfully nucleated in the bacterial cytoplasm [90]. Flies, worms, and plants also have Hsp104 homologs – it will be interesting to see whether these are also capable of propagating yeast prions or their own, endogenous PriLiPs. Mammals have no Hsp104 homolog and had been thought to lack disaggregase machinery, but recently Hsp110 has been shown to cooperate with Hsp70 and Hsp40 to this effect [91]. While the mammalian machinery was not able to remodel the yeast prion Sup35, it may yet have similar activity for PriLiMs in its native cellular context.

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Figure Legends:

Figure 1. Hypothesis: Bet-hedging prions () are adaptive and can respond to stress.

Yeast prion states provide advantages in a variety of environments [17], [18], and prion switching increases in response to environmental stress [35]. Two types of prion-switching induction are proposed – stochastic and specific. The "blue environment" signifies unpredictable environmental stresses in which prions are induced stochastically. This might be observed for any stress that significantly perturbs protein homeostasis and stresses the chaperone machinery involved in maintaining prion states. Note that each different prion causes a different phenotype, indicated by the color of the cell. After competition, a prion state that proved advantageous dominates the population of cells. The "green environment" signifies an environmental stress that induces a specific prion pre-adapted to enhance survival in that condition. This is more likely to occur for stresses that are encountered regularly throughout the evolution of the organism. Specific prion induction has been observed for [*MOT3+*] in ethanol (Halfmann and Lindquist, unpublished data), and for [*GAR+*] in the presence of bacterial competitors (Jarosz and Lindquist, unpublished data). Note that there will generally be a low frequency of appearance and disappearance of each prion state (not depicted).

Figure 2. Hypothesis: Bet-hedging prions allow rapid phenotypic diversification, acquisition of complex traits, and facile reversion to previous phenotypes

(A) Different combinations of prion/non-prion conformations amongst many available prion proteins allow shuffling of heritable phenotypes. The red and blue cells indicate two possible combinations of prions, and thus heritable phenotypes, between genetically identical cells in a population. A cell will switch to a new prion state at a rather low frequency. Thus, it is possible to generate new combinations of prion states that are not present or may have previously died out.

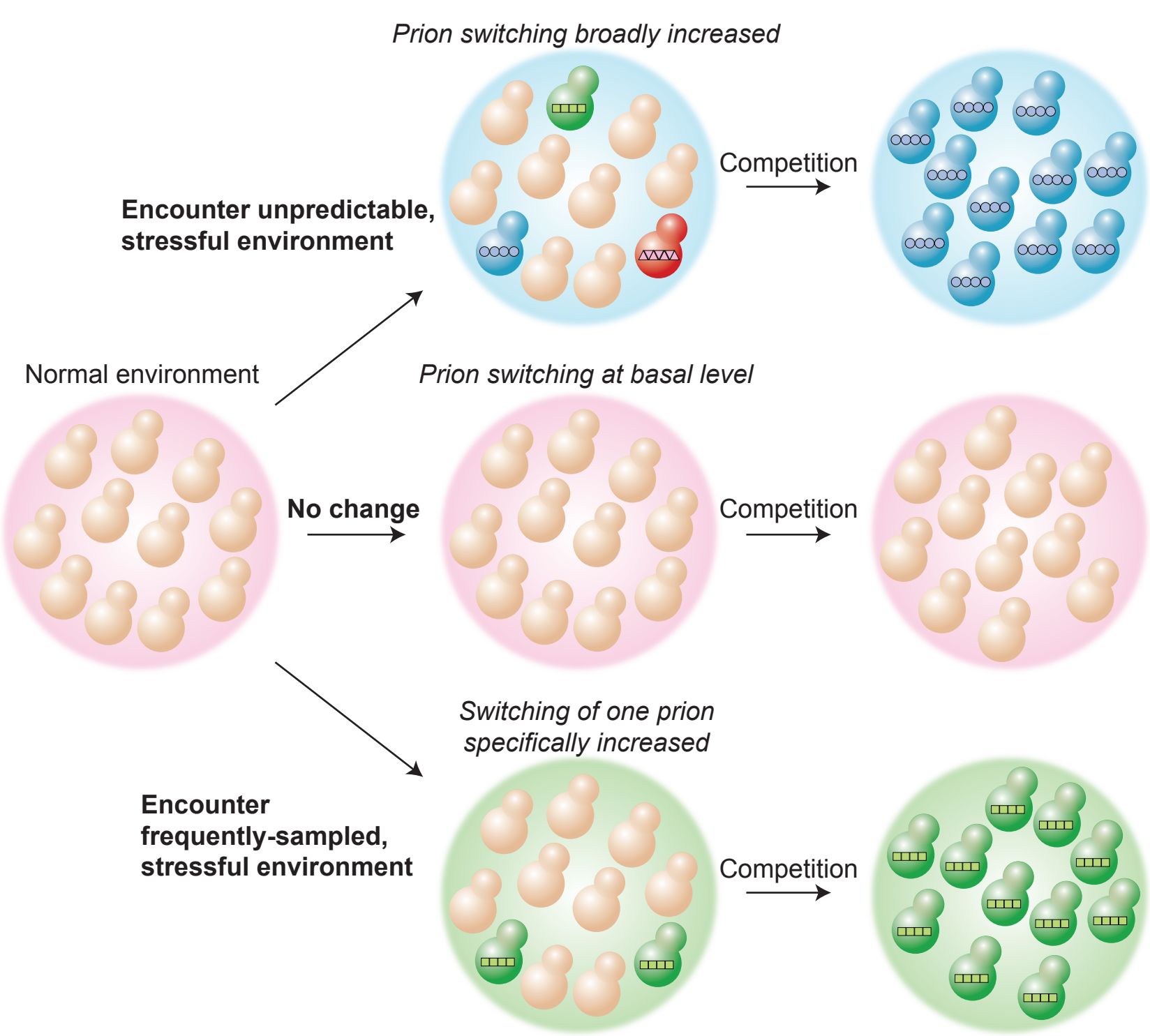
(B) Cells experience slowly-oscillating environments and may benefit from resampling phenotypes that were advantageous in the past. Adaptations made through bet-hedging prions are reversed more frequently than are mutations. This could allow cells to adapt to previously-encountered environments more quickly.

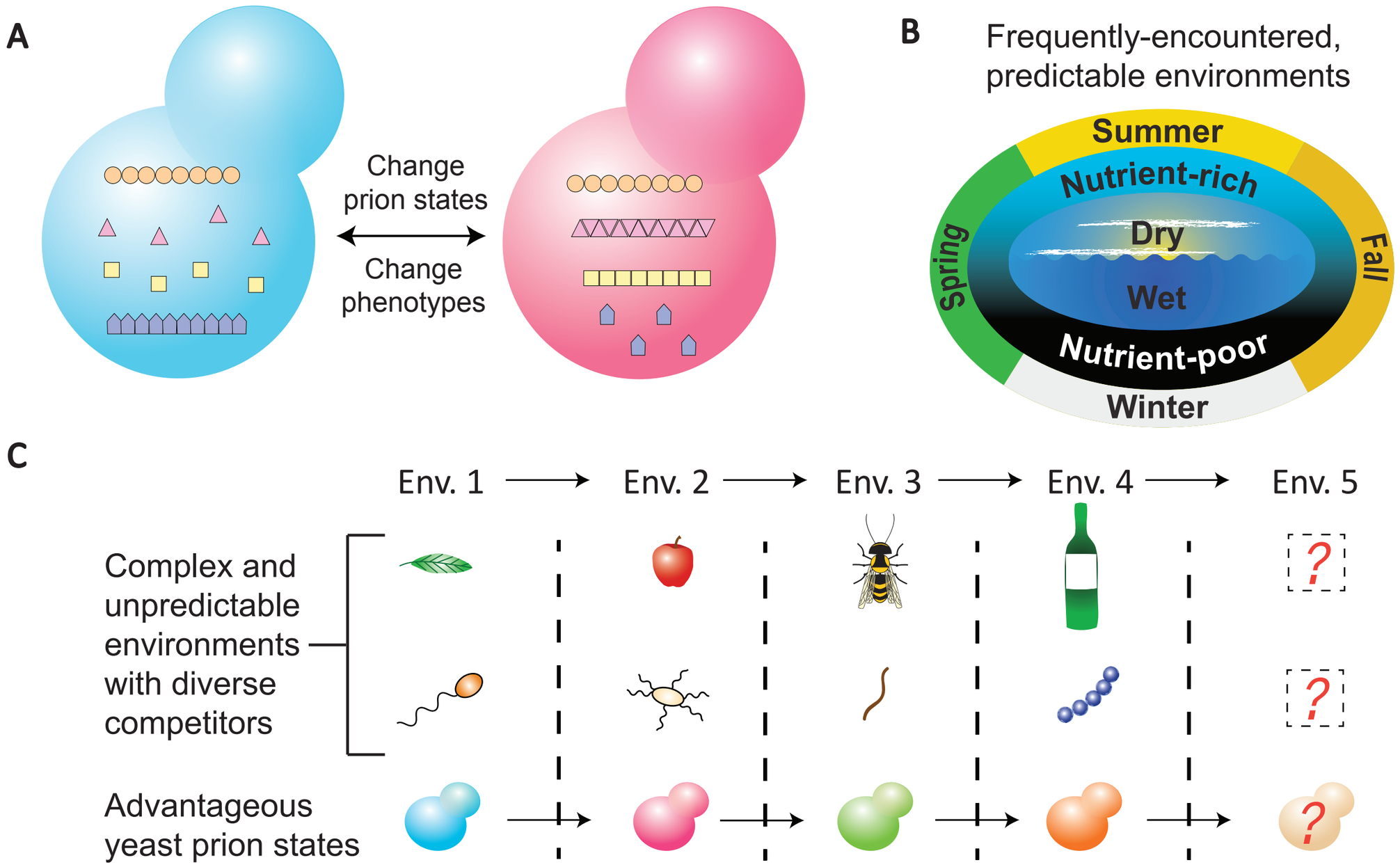
(C) Cells frequently sample new, complex environments, for example as different microbial competitors and surfaces are encountered. Shuffling the states of multiple prion proteins (indicated by different yeast cell colors) allows rapid phenotypic diversification enhancing the likelihood that some members of the population will adapt and survive each new environment. Here, the yeast sample environments progressing from leaf, to fruit, to insect, to liquid culture, each with its own set of microfauna, and different prion states dominate the population in each environment. In the next, unknown environment another combination of prion states may be advantageous. Many prion combinations may be present at a low frequency in the population prior to entering the environment, and the stresses of a new environment may induce additional prion switching to enhance adaptation.

729 **Figure 3. PriLiMs can alter the biological properties of a protein.**

730 (A) Prion-like assemblies may alter protein-protein interactions. Mitochondrial antiviral
731 signaling protein (MAVS), on the surface of mitochondria, interacts with TNF receptor-
732 associated factors (TRAFs) after prion-like aggregation [6].

733 (B) Other proteins gain catalytic function when they assemble into amyloid. Here, RIP1 and
734 RIP3 are depicted as inactive kinases that are activated upon assembly. This activity is thought
735 to be in part due to enhanced auto- and cross-phosphorylation in the assembled form, which is
736 prevented by other factors before assembly [67], [68]. The kinase image was adapted from
737 PDB entry 2J2I for purely illustrative purposes.


Prion switching broadly increased
Encounter unpredictable, stressful environment
Competition
Normal environment
Prion switching at basal level
No change
Competition
Switching of one prion specifically increased
Encounter frequently-sampled, stressful environment
Competition


A
Change
prion states
Change
phenotypes
B
Frequently-encountered,
predictable environments
Summer
Nutrient-rich
Dry
Wet
Nutrient-poor
Winter
Spring
Fall
C
Env. 1
Env. 2
Env. 3
Env. 4
Env. 5
Complex and
unpredictable
environments
with diverse
competitors
Advantageous
yeast prion states
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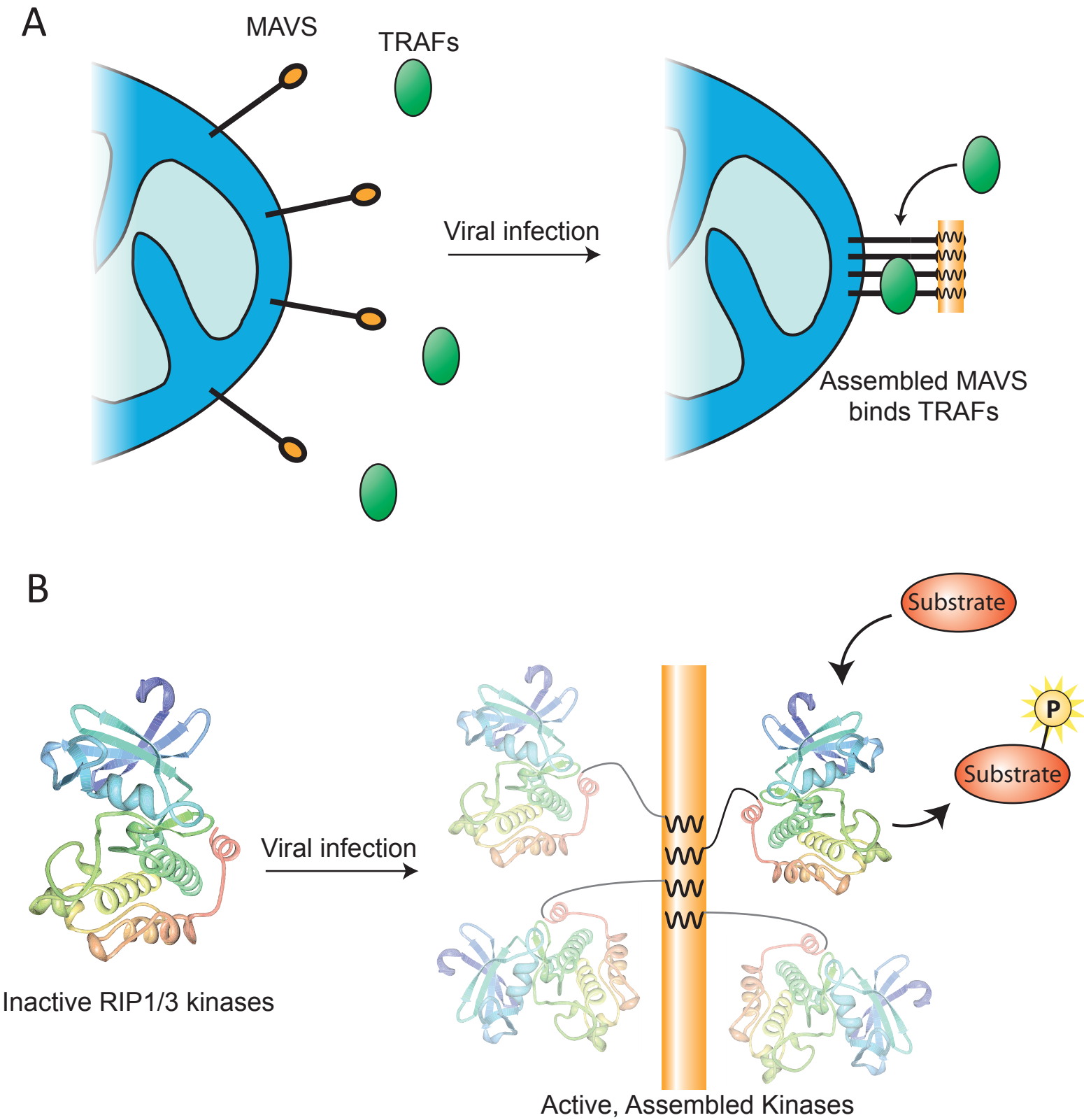
A
MAVS
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Viral infection
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binds TRAFs
B
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P
Substrate
Viral infection
Inactive RIP1/3 kinases
Active, Assembled Kinases