CHEMICAL REVIEWS

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Review

When Phased without Water: Biophysics of Cellular Desiccation, from Biomolecules to Condensates

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Cite This: *Chem. Rev.* 2023, 123, 9010−9035

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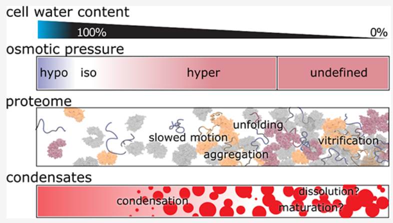
ABSTRACT: The molecular machinery that enables life has evolved in water, yet many of the organisms around us are able to survive even extreme desiccation. Especially remarkable are single-cell and sedentary organisms that rely on specialized biomolecular machinery to survive in environments that are routinely subjected to a near-complete lack of water. In this review, we zoom in on the molecular level of what is happening in the cellular environment under water stress. We cover the various mechanisms by which biochemical components of the cell can dysfunction in dehydrated cells and detail the different strategies that organisms have evolved to eliminate or cope with these desiccation-induced perturbations. We specifically focus on two survival strategies: (1) the use of disordered proteins to protect the cellular environment before, during, and in the recovery from desiccation, and (2) the use of biomolecular condensates as a self-assembly mechanism that can sequester or protect specific cellular machinery in times of water stress. We provide a summary of experimental work describing the critical contributions of disordered proteins and biomolecular condensates to the cellular response to water loss and highlight their role in desiccation tolerance. Desiccation biology is an exciting area of cell biology, still far from being completely explored. Understanding it on the molecular level is bound to give us critical new insights in how life adapted/can adapt to the loss of water, spanning from the early colonization of land to how we can deal with climate change in our future.



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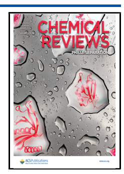
Received: September 25, 2022
Published: May 3, 2023



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https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

1. LIFE AS A FUNCTION OF WATER AVAILABILITY

Biology is a "wet" science: cellular metabolism can only occur when cells are hydrated. Organisms therefore must have evolved mechanisms to sense water availability and to respond to its limitation to ensure survival. Compellingly, certain organisms have evolved ways to temporarily survive desiccation, the near-complete absence of water, and are thus showing us the limits of biology. Looking at the enormous variety in ecosystems, organisms, and the strategies they use to survive desiccation indicates that the required water availability for one organism does not necessarily meet the standards of another (Figure 1). This biodiversity highlights the complex adaptations that organisms have evolved to thrive in their respective niches.

The ability of some organisms to survive desiccation has been studied for decades. We point interested readers to an excellent review on the progress made in the area of desiccation tolerance by Leprince and Buitnik.[1] It is not surprising that our progress in the field of desiccation walks hand in hand with the advance in biological techniques. Before the 2000s, findings were underpinned by biochemical studies that allowed us to understand that specific biomolecules and metabolic pathways correlate with the ability to cope with the state of desiccation.[2] After the start of this millennium, the boom caused by massive strides in DNA sequencing technology allowed us to take giant steps in molecular biology and comparative genomics. This is how, for example, studies comparing resurrection plants with their nontolerant counterparts permitted us to recognize those genetic traits associated with the phenotype of desiccation tolerance.[3,4]

Despite the progress made in understanding how water availability affects the viability of a select set of model systems, our current knowledge of the molecular mechanisms that underpin the ability to sense and adapt to changes in hydration is far from being fulfilled. Here, our goal is to survey our current understanding of these molecular mechanisms and highlight the physical−chemical concepts that unify them. We begin this review with a birds-eye view of how organisms might deal with limited water availability. We then discuss how dehydration affects cellular biochemistry. Given their outsized importance in desiccation protection, we describe how intrinsically disordered proteins (IDPs) can act as sensors of and responders to changes in water availability. Moreover, the newly found appreciation of their role in highly dynamic biomolecular condensates suggests that such compartments could act as important signaling hubs in times of water stress. Following this molecular-level focus, we review the current evidence implicating IDPs and protein condensates as responders to water availability at the cellular and organismal level and highlight how such insights may have far-reaching technological and agricultural implications, especially in light of climate change.

1.1. Water Stress

In the lifetime of many organisms, there comes a moment when water availability is limiting or may suddenly change. The first efforts to understand the effects of water limitation centered their attention on the plant kingdom's response. The large phenotypic and ecological diversity in this kingdom made it clear that water stress spans a broad spectrum, going from mild to moderate to severe stress and finally desiccation.[5]

In the same way that plants suffer from water stress, almost any organism could experience changes in water availability. Even small changes in cell volume lead to alterations in its water content, which could affect osmotic pressure and the concentration of its molecular components. While depicting the cell as a "bag of chemicals" is often overly reductive, this view can be instructive to think about what would happen to such a simplified cell when the environment surrounding it dries out.

As water is removed from the environment, the concentration of extracellular solutes increases. This causes a rise in extracellular osmotic pressure. Any biological barrier, be it a lipid membrane, a cell wall, or any other mechanism that isolates the cell's contents from its surroundings, must be permeable; life cannot exist without the transfer of material into and out of the cell. As such, the osmotic pressure building up in the exterior will be felt in the cell's interior. The cell must allow for the flow of water through its membrane to ensure osmotic equilibrium and prevent damage to the cell's membrane. This is often done through passive channels such as aquaporins[6] that allow the rapid flow of water molecules. In turn, this decreases the concentration of cellular solutes and returns them to their normal levels. Water flow will continue until the intracellular osmotic pressure is equal to that on the outside.

As water leaves the cell, the concentration of cellular solutes increases. This is because even though solutes can also efflux out of the cell (actively or passively through channels and transporters), the rate at which this happens tends to be orders of magnitude slower than that of water.[7] The cytoplasm, previously with enough water to ensure optimal biomolecular behavior and function, now becomes crowded and more viscous. As more water leaves the cell, the average distance between biomolecules decreases, causing an increase in their interactions. This can lead to aggregation and precipitation that is often irreversible. Thus, when reaching this state, even upon the reintroduction of water, the cell can no longer recover and fails to survive.

1.2. Anhydrobiosis

Depending on the environment they live in, organisms have evolved specific strategies to cope with water limitation (Figure 2). Examples that come immediately to mind are cacti and camels, both of which are exquisitely adapted to desert environments. Despite the obvious differences between them, both organisms exemplify how water limitation can be tolerated by decreasing water loss and concomitantly increasing water uptake and storage in times of abundance. While such organisms (xerophiles) can thrive under conditions of low water availability, by no means do their cells hold less water than their nondesert counterparts. However, across the Tree of Life we do find organisms that approach the biological limits of cellular life and undergo a state of so-called *anhydrobiosis*.

Anhydrobiotic organisms can completely dry out and stay dormant in a metabolically quiescent state for prolonged periods of time that can extend to decades, even centuries, in some cases. Such extreme desiccation tolerance (DT) is defined as the ability of organisms to equilibrate their water content with that of the ambient environment and subsequently return to normal activity upon rehydration.[8]

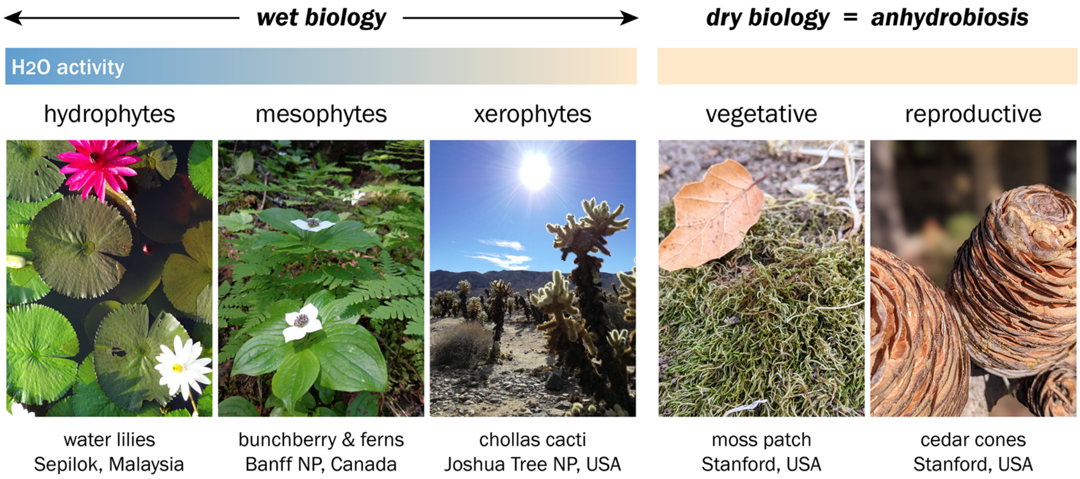

Figure 1. Examples of wet versus dry biology in the green plant Kingdom. (left) Examples of plants adapted to environments with different water activity (hydro, high; meso, moderate; xero, low). Despite the difference in the availability of water, these plants are hydrated and able to maintain enough water to sustain metabolic activity. (right) Certain plants have evolved the ability to temporarily survive complete desiccation, characterized by loss of most cellular water and a halt in metabolism. This can be observed for both vegetative stages, so-called resurrection plants, or specialized reproductive propagation stages, such as seeds and pollen.

While the concept is intuitive, defining the physiological threshold under which the organisms can be considered dry is not. One definition that is often used as a standard is "the ability to survive drying to, or below, the absolute water content of 0.1 g H_2O/1 g dry mass, this being equivalent to air-dryness at 50% relative humidity and 20°C and corresponding to a water potential of ≤ -100 MPa".[9] This definition was chosen based on historical reasons, but also because it coincides with the threshold under which there is not enough water to form the needed monolayer around macromolecules and membranes for enzymatic activity to occur.[10] Remarkably, despite the fact that water loss should theoretically induce various types of cellular damage such as membrane destabilization, protein denaturation, and oxidative stress, DT organisms can resume their life cycle upon rehydration.[11]

DT is found throughout the Tree of Life,[12,13] and broadly follows two strategies. First, organisms can induce biochemical changes that make them desiccation tolerant in times of water limitation, after which they resume growth upon recovery after rehydration. Second, several organisms pursue active desiccation to generate stress-resistant propagation vectors. Most studies on DT have been conducted in plants because most organisms in this kingdom exhibit at least one form of DT. In more ancient clades such as algae, mosses, liverworts, and hornworts, tolerance is typically "constitutive," meaning nonreproductive tissues respond to their surroundings by equilibrating water content with the atmosphere. In this scenario, rapid drying dictates DT, and desiccation recovery is subjected to the response of undamaged tissue after rehydration.[14] For many higher plants, DT is an essential part of their development. This trait is embodied by reproductive vectors such as seeds, pollen, and spores, which allows them to travel in both space and time by only reactivating when and where conditions are right.[15−17] Although rare, vegetative tissues of some higher plants can also show DT.[3,18] In this scenario, vegetative tissues must undergo a gradual water loss, leading to an "induced" response that allows them to survive desiccated conditions.[19]

Although less studied outside of the plant kingdom, we do find that other organisms can also pursue both of these DT strategies. In the animal kingdom, the brine shrimp (*Artemia*),[20] sleeping midge (*Polypedilum*),[21] *Aedes* mosquitoes,[22] and the nematode *Caenorhabditis elegans*[23] can acquire DT during embryonic and early larval stages, respectively. These organisms arrest their development if the environmental conditions are nonoptimal and resume it when the conditions become favorable. On the other hand, several tardigrades[24] and rotifers[25] survive desiccation in adult stages when drying occurs gradually. Among microbes, both prokaryote and eukaryote ones, DT is common.[26−28] Bacterial and yeast spores are in ways similar to plant seeds, as they present propagation vectors that can withstand several stresses and aging. But even their vegetative cells can survive desiccation, as illustrated by the industrial production of dried baker's yeast. The same goes for protists, which often form DT cysts in times of suboptimal growth conditions.[29] It is remarkable that despite the distances in the evolution between all of these anhydrobiotic species, they share similar mechanisms to tolerate desiccation. We discuss these mechanisms in sections 3 and 4 of this review.

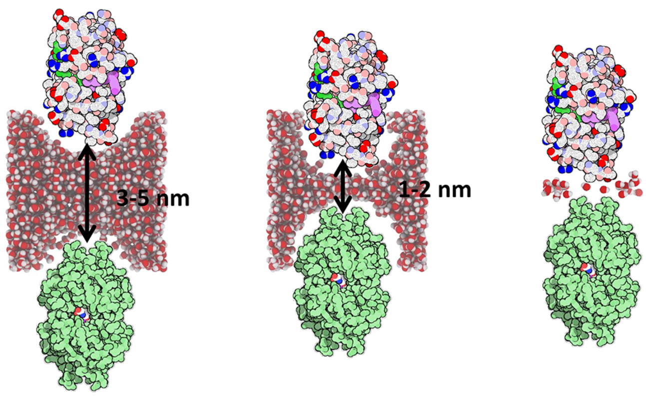

Figure 2. Schematic illustration of the water molecules separating two proteins in a well hydrated, dehydrated, and desiccated cell. This cartoon does not include other small molecules which might exist at high concentrations, especially under desiccation.

Box 1. How many water molecules between two proteins?

Yeast cell volume:[47] $50\ \mu m^3 = 5 \times 10^{14}\ \frac{L}{cell}$

Yeast protein copy numbers:[48] $70 \times 10^6\ \frac{proteins}{cell} = 1.16 \times 10^{-16}\ \frac{mol}{cell}$

Protein molar concentration in the cell: $\frac{1.16\times10^{-16}\frac{mol}{cell}}{5\times10^{-14}\frac{L}{cell}} = 2.3\ mM$

Average distance between two proteins center of mass (a function of concentration):[49] $1.18 \times 0.0023\ M = 9\ nm$

Average radius of a protein:[50] $\sim 2\ nm$

Average distance between two protein surfaces: $9\ nm - 2 \times 2\ nm = 5\ nm$

Diameter of a water molecule: $0.3\ nm$

Number of waters between two proteins: $5\ nm\ /\ 0.3\ \frac{nm}{water\ layer} = 16\ layers$

1.3. Metabolic Response to Water Loss

Water stress responses vary according to an organisms' adaptation to water constraints. Desiccation-sensitive organisms are unable to adjust their metabolism, resulting in a deathly fate. On the other side, anhydrobiotic organisms are able to withstand desiccation by progressively adjusting (or "priming") their metabolism to transform their cellular activity and internal environment until reaching a dormant tolerant state. If this priming does not occur, the organism is generally unable to survive desiccation. In the case of tardigrades, for example, if acclimation, consisting of a slow and progressive dry, does not happen, desiccation can be lethal.[30,31] *Saccharomyces cerevisiae* displays a similar phenotype, where robust desiccation tolerance is achieved only after extended exposure to starvation in saturated cultures.[32,33] This biochemical priming involves a complex orchestration of numerous genes that modulate carbon flux, respiration, the oxidative stress response, and the expression of effector proteins like chaperones or late embryogenesis abundant (LEA) proteins.[1]

Anhydrobiotic organisms do not just switch off their carbon metabolism to enter into a dormant phase but first deviate carbon flux into the synthesis of osmoprotectant metabolites. For example, organisms like yeast, some tardigrades, brine shrimp, and the nematode *C. elegans* tune their carbon flux to synthesize trehalose and glycerol.[1,34,35] Meanwhile, resurrection plants and orthodox seeds (i.e., seeds that completely dry out) allocate carbon use to the synthesis of nonreducing oligosaccharides, such as sucrose and raffinose.[36] Lastly, *Aedes* mosquito embryos subjected to water stress invest in the production of polyamines as osmoprotectants.[22] These often organism-specific metabolic and biochemical changes act in conjunction with the drying cellular environment to alter the cell's (bio)chemical composition, both for abundant, low molecular-weight compounds and for larger biomolecules including proteins and nucleic acids.

A major cellular problem associated with dehydration is linked to aerobic metabolism. Water stress unbalances respiration, leading to the overproduction of reactive oxygen species (ROS). In photosynthetic organisms, this poses an additional challenge as the photosynthetic machinery constitutes a rich source of ROS.[37] Free radical accumulation, further enhanced by the overconcentration due to water loss, causes serious impairment to cellular components and damages phospholipid membranes, nucleic acids, and proteins.[38,39] Therefore, a conserved metabolic response in DT organisms is the upregulation of the oxidative stress response.[40]

To withstand the increase in ROS, DT organisms upregulate free-radical processing enzymes like superoxidase dismutase (SOD), glutathione reductases, glutathione peroxidases, and catalases, which all control the buildup of free radicals, hereby preventing oxidative damage.[41] Besides enzymes, DT organisms can additionally accumulate antioxidant molecules, such as glutathione and ascorbate.[40,42] The actual entry into the dormant phase is also helpful, as this process coincides with a deacceleration of respiration, which drops oxygen consumption and limits the production of ROS. What is remarkable though is that the enzymes involved in this oxidative stress response are not specific to anhydrobiotic organisms but fairly universal. So, the ability to tolerate desiccation by these organisms must involve unique, yet unclear, mechanisms that trigger the upregulation (and perhaps protection) of these enzymes and other effector proteins during dehydration.

2. BIOPHYSICAL CHEMISTRY OF WATER STRESS

Inside the cell, biomolecules are immersed in a dynamic and heterogeneous aqueous environment. In such an environment, ions, small solutes, metabolites, and macromolecules diffuse rapidly and are taken up and being released, synthesized and degraded. Coupled to rapid, size-dependent diffusion inside the cell,[43,44] this complex environment can display temporal and spatial fluctuations in copy numbers, concentrations, and global physical parameters such as pH and ionic strength. Water makes up 60−70% of the cell's mass and plays a critical role in the function of all biomolecules. Indeed, all biomolecules subjected to evolutionary pressure and playing a functional role in the aqueous cellular environment have evolved to function optimally in a specific range of water content. Strong deviations from this range can result in a catastrophic failure of their function.

As the environment around the cell dehydrates, water exits the cell to reduce the osmotic pressure across the cellular membrane. When cellular water effluxes out of the cell, the intracellular environment becomes dramatically different. To illustrate this, we will use a eukaryotic yeast cell, *S. cerevisiae*, as a model to get a rough estimate of what happens in a single cell under water stress. In a yeast cell, total protein concentrations average around 2 mM, which means that two proteins are separated, on average, by only 10−20 layers of water (see Box 1). During water stress, a significant portion of this water leaves the cell.[45,46] We classify two main challenges to biomolecules that arise due to this effect: a thermodynamic challenge and a kinetic challenge, and discuss both below.

Box 1

2.1. Thermodynamic Forces during Water Stress

As water leaves the cell, the concentrations of all solutes, which can take orders of magnitude longer to diffuse into or out of the cell compared to water,[7] increase dramatically. Such a change in composition carries with it an increase in macromolecular crowding, as well as a change in the interactions between the small and large molecules that make up the cell. This alters the thermodynamics of the system, causing molecules to adapt their structure and interact and aggregate in ways that would not occur in a fully hydrated state. The driving force for these effects can be both enthalpic and entropic in nature (and almost always a combination of both). Generally speaking, for the cases we are concerned with, enthalpic forces are those that result from the noncovalent interactions between the cellular solutes. These depend on the chemistry of the constituents. Entropic forces arise from factors where the chemistry is irrelevant: mixing or translational and rotational degrees of freedom. This breakdown is very rough, and of course there is often coupling (or compensation) between these two terms.[51] Nonetheless, it provides a useful way to discuss the effects of dehydration through the lens of thermodynamics.

2.1.1. Entropic Forces during Water Stress. An entropic driving force is one that depends primarily on concentrations and molar volumes rather than on attractive or repulsive noncovalent interactions.[52,53] Water stress in biological systems changes the concentration of solutes, increasing what is referred to as macromolecular crowding[54] or depletion force.[55] This entropic force arises from the volumetric constraints enforced by large solutes in the cellular milieu (see Boxes 2 and 2a). As water leaves the cell, the concentration of solutes increases. By assuming more compact conformations, by associating into homotypic or heterotypic oligomers or by aggregating (referring here to all processes in which the end state takes up less volume), a protein frees up space for other diffusing molecular species. This increases the system's overall entropy, driving down the free energy of the system. The higher the solute concentration, the larger the contribution of volume reduction, and the more entropy increases due to this volume compaction. This effect has been observed in countless *in vitro* experiments[56−59] and also inside cells.[60−62] However, decoupling the contribution of this force from others becomes problematic to measure as the solvent, in this case water, becomes scarcer. This is both because repulsive and attractive interactions become dominant contributions to the thermodynamic state of the protein (due to an increase in its concentration and the scarcity of water), and the fact that free movement within the cellular milieu is impeded.

2.1.2. Enthalpic Forces during Water Stress. The second thermodynamic driving force for the effects of water stress arises from the interactions between biomolecular surfaces and the solvent and solutes that surround them.[52,64,65] Unlike macromolecular crowding, which is entropic in nature, interactions are enthalpic and can be either attractive or repulsive. A classic example for this is electrostatic interactions, where two oppositely charged surfaces will induce an attractive interaction, but two like-charged surfaces will repel each other. Perhaps due to the importance of this effect,

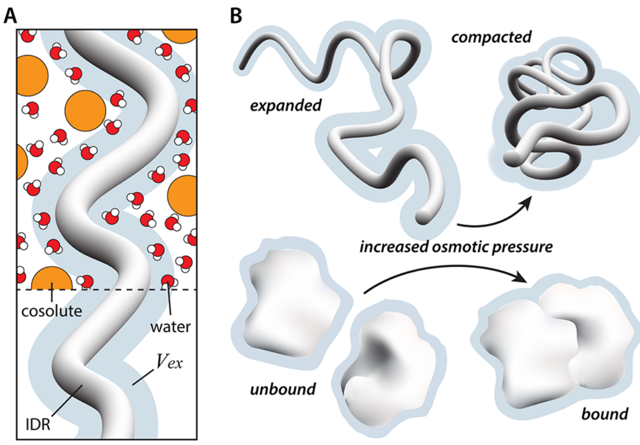

Figure 3. Osmotic pressure exerted by molecular crowding. (A) In the cartoon above, water molecules can freely diffuse in and out of the blue bound region surrounding a disordered protein (IDR), while the center of mass of the larger cosolutes (orange) are sterically blocked from entering. This effectively creates an osmotic pressure, $\Pi = cRT$ where c is the cosolute concentration and R and T are the universal gas constant and the temperature in K, respectively. Increasing cosolute concentration will increase Π linearly. The free energy cost of transferring the biopolymer from pure water to a cosolute solution is given by $\Delta G_{tr} = \Pi V_{ex}$, where V_{ex} is the volume of the blue region, determined by the effective radius of the cosolute. Larger solutes will have a larger V_{ex}, increasing ΔG_{tr}. (B) An increase in osmotic pressure will drive molecules, such as both disordered and folded proteins, to minimize their respective V_{ex}.

the net charge of most proteomes is negative at typical physiological pH, preventing spurious self-association.[66] As the cell's water content drops, the concentration of small, but relatively abundant solutes (such as ATP, sugars, and other abundant metabolites, usually in the range of 1−10 mM) can increase by an order of magnitude. This changes abiotic cellular conditions such as the ionic strength and modulates the interaction between protein surfaces and the small molecules surrounding them.

The effects of water stress extend beyond just a change in concentration, however. The water molecules immediately surrounding the surface of a biomolecule are ordered differently from pure (or "bulk") water.[67] This effect extends 2−4 layers of hydration away from the protein[68−70] (although some reports of even longer ranges exist[71,72]). At ∼10−20 layers of water between two proteins, there is enough water to form extended hydrogen-bonded networks that at least have a semblance to pure water (Figure 4A).

However, in a dry state, the number of water layers is drastically reduced, affecting the orientation, energetics, and dynamics of the intracellular water network.[73] In this state, no bulk water exists, and biomolecules must contend with a drastically altered chemical environment that has little semblance to the aqueous environment they were evolved to function optimally in (Figure 4B). This also dramatically differs from the aqueous solutions in which we perform most biochemical or thermodynamic studies of biomolecular systems. One main reason for this is the heterogeneity introduced by desiccation into otherwise homogeneous systems.[74] This heterogeneity interferes with the interpretation of average system properties.

Whether through an entropic or enthalpic mechanism (or most likely a mixture of both), the effect of desiccation on the

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

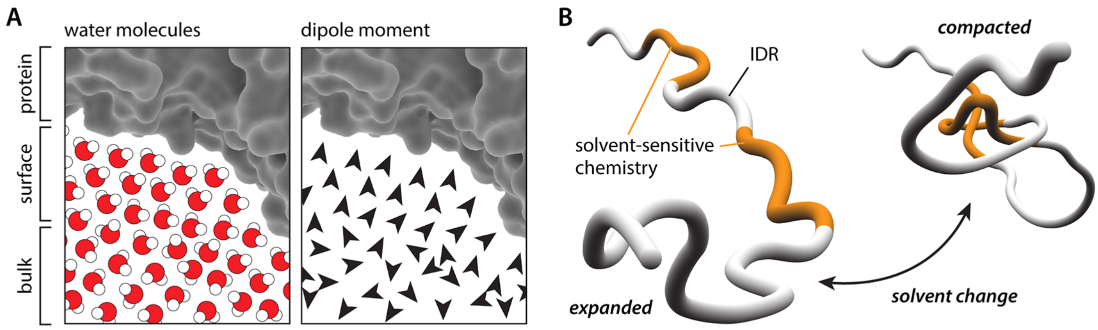

Figure 4. Enthalpic effects of biomolecular solvation. (A) Water forms hydrogen bonds with biomolecular surfaces, creating a preferential orientation at the interface. This preferential orientation decays further away from the interface (where water molecules act like pure or "bulk" water). (B) Biomolecules are generally not homopolymers and have different surfaces. Furthermore, the surfaces exposed to the solution depend on the conformations which the biomolecule assumes. Shown here are two conformations for a disordered protein exposing different chemistries to the solution.

thermodynamics of proteins and other large biomolecules is dramatic and often catastrophic. As a result, organisms that have not evolved (or have lost) mechanisms to mitigate water stress experience an irreversible failure in their viability when they encounter it. Indeed, most multicellular eukaryotes cannot recover from severe exposure to water stress even after water is reintroduced into the system. Yet, how this failure occurs at the level of a single cell, or even on the molecular level, remains poorly resolved.

2.2. Kinetic Effects in Desiccating Biological Systems

In many chemically heterogeneous systems, the rapid removal of solvent will cause the system to vitrify. The vitrified state of cells is a composite material where solute molecules exist in a solid-like mixture.[75,76] Nearly all living systems will reach this vitrified state if dried rapidly enough.[77] In such a state, motion is slowed to a halt, and reactions and interactions are all but impossible. Because of this, a prevailing thought was that vitrification is essential (and in some cases sufficient) for anhydrobiosis.[78] But while nearly any living system can be vitrified, only a fraction can survive this process. Many cells and organisms simply will not recover from desiccation, whether it occurs rapidly or gradually. Other organisms, even those able to withstand extreme desiccation, usually require that the loss of water occurs over a span of time in which they prime their biochemistry.[79,80] If such organisms dry too rapidly, they cannot survive the absence of water despite reaching vitrification.[32,81]

The effects of vitrification on the kinetics of biomolecular processes in living systems are relatively poorly studied in the context of anhydrobiosis. In essence, water can be thought of as a lubricant that facilitates biochemistry.[82] In its presence, biopolymers fold and function optimally, and diffusion of small and large molecules occurs at a fixed and rapid rate. As water is removed from the system, diffusion slows down and the rearrangement of biopolymers is hindered. If this process occurs sufficiently slowly, the cell has a chance to mitigate it by taking up or synthesizing solutes and larger biopolymers. If not, the cell's water is removed until just a few water layers remain between solutes in the cell (see Box 1). In this state, thermodynamic forces are pushing larger biomolecules closer together (through increased crowding), solute concentrations skyrocket, and eventually, the system reaches a vitrified state.[83] At this stage, and in the absence of machinery and/or molecular composition to prevent it, the mass of aggregated small molecules and biomolecules becomes inextricable. To survive anhydrobiosis, organisms therefore must prevent this irreversible process from occurring, at least for the critical components of their biochemical machinery.

The chemical composition of the cell is key to ensuring that the vitrified material can rehydrate successfully.[32,84,85] While many organisms, including the cells of all mammals, cannot survive desiccation at all, others can if they undergo slow desiccation coinciding with priming. Examples include prokaryotes,[28] yeast,[35] tardigrades,[30] roundworms,[86] and plant seeds.[83] Remarkably, some organisms are capable of surviving desiccation even if it occurs rapidly (e.g., moss), suggesting they have evolved a proteome that is capable of surviving vitrification at any point or have a chemical composition that is already in a "primed" state.[87] The exact chemical and physical differences between these three different types of cellular environments remains unknown.

3. BIOMOLECULES UNDER WATER STRESS

As water is removed from an organism, the concentrations of cellular solutes change dramatically.[88] Furthermore, the composition of these solutes can also change due to the cellular adaptation mechanisms that kick in to counteract water stress.[89−91] In the context of the thermodynamic driving forces mentioned above, it is important to note that the entropic force of macromolecular crowding is solely dependent on concentration and only acts to compact and bring molecules together.[92] The enthalpic effect, however, is affected by both changes to composition and concentration and can act not only to compact/aggregate but also to expand and drive dissociation of molecules. This occurs through the introduction of repulsive (compacting/aggregating) or attractive (expanding/dissociating) enthalpic interactions between the solute and the surface of the macromolecule. In *in vitro* experiments, the interaction between large biomolecules like DNA or proteins and specific solute environments has been quantified thermodynamically by measurements of transfer free energy (ΔG_{tr}, see Boxes 2 and 2a).[93−95] While these experiments provide a critical framework to understand the interactions between macromolecular surfaces and their surrounding environment, they become extremely difficult to interpret in an actual cellular environment. Below, we cover several classes of molecules and how they may be affected by water stress.

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

Box 2. Connecting osmotic pressure, crowding, and water content

From a physical−chemical perspective, the loss of water in an aqueous system can be defined in several ways. In introductory chemistry textbooks, the concept of osmotic pressure is one of the first to be introduced. Much like crowding, osmotic pressure is a purely entropic effect: the chemistry of the different species in the system should not, in theory, affect its magnitude directly. Instead, this is determined only by the concentrations of dissolved species in the solution. The effects of osmotic pressure, often described as a concentration difference across a water-permeable membrane, exist for all organisms. This is because the cell membrane or wall can be thought of as such a semipermeable membrane. However, osmotic pressure need not occur through a semipermeable membrane. Osmotic pressure can occur simply as a result of having molecules of different size in the same solution. In the example illustrated in Figure 3, a biomolecule (gray, IDR) is immersed in a solution containing water molecules (red) and a large cosolute (orange). The cosolutes are excluded from the surface of the biomolecule simply through a steric repulsion of their center of mass, creating an exclusion zone that is accessible only to the solvent molecules (blue shaded region). This exclusion zone has volume (referred to as the "excluded volume") that is determined both by the average radius of the cosolute and by the accessible surface area of the biomolecules. The free energy cost of creating this excluded volume (which is the same as the cost of transferring the protein from pure water to this cosolute solution) can be quantified through the so-called transfer free energy:

$$\Delta G_{tr} = cRTV_{ex}$$

where c is the concentration of the excluded cosolute(s), R is the universal gas constant, T is the temperature in K, and V_{ex} is the excluded volume. In this equation, cRT is simply the osmotic pressure exerted by the cosolute solution. This provides a direct link between crowding and osmotic pressure: increased cosolute concentrations amount to increased crowding, and this increases the free energy cost of creating the excluded volume V_{ex}. In other words, the increased osmotic pressure causes biomolecules to assume more compact conformations in order to reduce V_{ex}. For a more in-depth treatment of this equivalence, see ref 63.

3.1. Ionic Species

Ionic species, particular monovalent cations such as K^+, are the most abundant solute species in the cell and exist at concentrations exceeding 100 mM in normal conditions.[96,97] Ions can change their concentration by several folds under water stress.[98,99] This is enough to screen out nearly all intra- or intermolecular electrostatic interactions.[100−102] Electrostatic screening diminishes the attraction between oppositely charged macromolecules, which can inhibit misfolding and aggregation. However, screening also affects repulsive interactions between like-charged surfaces which may prevent aberrant interactions. In this case, the presence of high electrolyte concentrations promotes aggregation. It is well-known that proteomes tend to contain a net negative charge, possibly in order to prevent electrostatically driven aggregation.[66,103] Increased screening, therefore, is likely to increase the tendency of the proteome to aggregate. Furthermore,

Box 2a. Osmotic pressure, osmotic potential, water activity: negative or positive, high or low?

Different fields look at osmotic pressure from different angles. The osmotic pressure (commonly symbolized Π) is the pressure exerted on water by the presence of a solute and is directly proportional to the concentration of solutes. Osmotic potential (commonly symbolized Ψ, and also known as solute potential) relates to the potential of the solute to cause water movement, and has the same units and magnitude of the osmotic pressure, but is always negative. Water activity (commonly symbolized a_w) is the normalized vapor pressure above an aqueous solution. For pure water $a_w = 1$. For any aqueous solution, the vapor pressure will be lower than that of pure water so water activity will be lower than 1, but cannot be lower than 0.

When a cell loses water, the osmotic pressure of the intracellular environment increases, the osmotic potential decreases (becomes more negative) and the water activity decreases (moves closer to 0). In this manuscript, we primarily discuss osmotic pressure but remind the reader that these measurements are all linked and report on similar properties.

divalent ions, which play specific roles in enzymatic activity, signaling, and other cellular functions, nominally exist at lower concentrations. At higher concentrations (generally above 0.5 M for monovalent ions), ions begin to exert their effect through specific interactions.[104,105] Unlike screening, these interactions affect biomolecules in a way that depends on the identity of the ion and its interaction with the biomolecular surface. While some specific ions such as sulfate can strongly promote biomolecular aggregation (often referred to as "salting out"[106]), the most prevalent ions (K^+ in most eukaryotes) are relatively benign in this respect. Still, other ionic species, for example those containing phosphate groups, can interact strongly with different biomolecular species, driving different behaviors. Of note is the effect of ATP, which has been shown to inhibit protein aggregation by potentially acting as a hydrotrope.[107] Other, less abundant ions are also known to promote dysfunction at the protein level.[108,109]

3.2. Osmolytes and Other Small Molecules

While their concentrations tend to be lower than monovalent ions, metabolites and other small molecular species in the cell can exist in mM concentrations, although the range of these concentrations varies wildly between organisms and molecular species. Organisms across all kingdoms of life change the composition of their small solutes under water stress, often by orders of magnitude, and in an active way.[110−112] Such solutes are commonly referred to collectively as osmolytes and include free amino acids, sugars and polyols, and amine derivatives.[67,96,113] Osmolytes are biosynthesized or actively taken up from the environment to restore optimal physical chemical conditions following the initial osmotic shock response of the cell.[90,91,111,114] These cosolutes tend to work ubiquitously on the cell's macromolecular machines, increasing thermodynamic stability,[101,115,116] modulating cellular viscosity and kinetics,[117−119] and buffering out deleterious effects that may destabilize the proteome.[120] The mechanism by which this occurs is generally driven by enthalpic interactions with protein and nucleic acid backbones.[101,121,122] Remarkably, osmolytes are no one-size-fits-all solution. Different organisms accumu-

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

late specific osmolytes during dehydration or desiccation, creating a specific intracellular environment enriched in one specific type of osmolyte. For example, while plants commonly synthesize or take up the sugar sorbitol as they prime for dehydration, yeast prefer to use trehalose.[32,123−125] This points to the importance of specific solute−biomolecule interactions in preserving biological function under water stress.

3.3. DNA/RNA

It is common knowledge that DNA is resistant to desiccation; after all, we share DNA plasmids on dry filter paper with little effect on their integrity. Maybe it is for that reason that few studies have been dedicated to understanding the molecular effects of water stress on DNA. Habitually, the DNA helix exists in the canonical B conformation (right-handed), and water molecules play a major role in its stabilization. Because of that, it has been suggested that water stress could alter B-DNA structure, favoring a transition into the less common A (right-handed) and Z (left-handed) helical conformations.[126] Moreover, water stress could also modify the supercoiled state of DNA molecules.[127] The fact that water availability dictates DNA conformation becomes even more important if we consider that DNA−protein interactions are governed by structural recognition, and therefore any activity inside the cell that involves DNA−protein interaction could be reshaped during water stress.

When water leaves the cell, the crowding and excluded volume effect could modify the interactions and conformations of nucleic acids. *In vitro* experiments showed that the presence of PEG, which imitates the effect of molecular crowding, favors more compact RNA structures.[128,129] Studies have shown that crowding agents can indeed change nucleic acid behaviors, for example, accelerating ribozyme-mediated catalysis or enhancing transcription rates.[130,131] But what is the consequence of dehydration on nucleic acids in the cell's interior? It is known that organisms exposed to water stress halt DNA synthesis, impacting cell replication, transcription, and protein synthesis.[132−134] One of the best characterized effects of water loss on DNA is the damage triggered by ROS accumulation[135−137] (see also section 1.3). In the same vein, RNA damage from desiccation is also poorly explored. A survey of seeds from different organisms stored over several decades reveals that RNA is degraded over time, and this effect correlates with the seeds' ability to germinate.[138] Yet, the mechanism by which this degradation occurs, or whether it differs in desiccation-sensitive organisms, is unresolved.

3.4. Lipid Bilayers

Lipid bilayers play an existential and defining role in every cell by creating a semipermeable, multifunctional barrier separating the cell from the extracellular environment and delineating specific organelles (e.g., lysosomes, mitochondria) from the cell's cytoplasm. Damage to lipid bilayers can irreversibly damage cells by affecting key internal homeostatic processes or disrupting the plasma membrane (Figure 5). At the molecular level, most lipid bilayers contain a polar or charged headgroup, which is solution-facing (i.e., outside of the bilayer interior) and forms strong attractive interactions with water.[139] As dehydration sets in, water layers are removed, the diffusion of coordinated water along the bilayer slows, and the properties of the bilayer change in tune.[140] In such a desiccated state, lipid membrane fusion and rupture are observed across a range of cells and organisms.[33,140−142] The underlying causes of these vary and may differ between different circumstances and

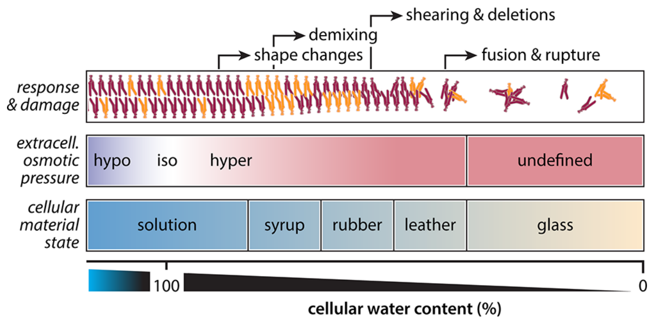

Figure 5. Lipid bilayers under water stress. As the water content in the cell is lost, the properties of membranes are altered. As osmotic pressure sets in, water leaves the cell causing membrane to fold and morph. The properties of the membrane change as further water stress occurs, with demixing of lipids and shearing and deletion within bilayers. Finally, fusion and membrane rupture cause irreversible damage in many cell types.

organisms. It has been hypothesized that membrane dysfunction may be caused by depletion of the water layer associated with the lipid headgroup and the subsequent stiffening of the bilayer.[143,144] The reduction in cell volume and the changes in cell morphology cause physical changes to bilayer properties that renders them susceptible to breakage and rupture. Like nucleic acids, changes in lipid properties could also be the result of an increase in ROS, which can oxidize membrane lipids and thereby change their properties.[145]

A general idea is that anhydrobiotic and DT organisms have the ability to either prevent or repair their membrane upon rehydration. The first is through the interaction between the membrane and protective cosolutes or osmolytes. Several studies have shown that a subset of protective solutes interacts directly with lipid membranes,[146] or alternatively, traps water next to the bilayer headgroups.[122] Whether direct or indirect, this association alters the properties of lipid membranes, presumably expanding their fluidity range and helping prevent membrane fusion or rupture.[146] The exact mechanism by which this occurs, however, remains unknown.

3.5. Proteins under Water Stress

As the molecular machines that carry out most cellular functions, proteins are key constituents of the cell that need to be able to survive desiccation. Multiple classes of proteins related to stress mitigation, and anhydrobiosis specifically, have been characterized.[67] These include chaperones and heat shock proteins that are able to disaggregate and refold the proteome following desiccation stress,[84,147] reductases that counteract the effect of reactive oxygen species[41] and specialized disordered proteins that help the cell survive anhydrobiosis[148,149] (see also section 4). But what are the driving forces for protein malfunction during desiccation?

Unless water stress is a routine event in a cell's environment or organism's lifestyle, there is no strong selective pressure to evolve proteins that maintain solubility under extreme desiccation. As described previously, removal of water from living systems brings solute concentrations to extremely high levels. This causes strong interactions between solutes and proteins that can be broken down into two types of effects: the first one is intramolecular and involves the loss of the three-dimensional structure of a protein that is required for most of them to function; the second one is intermolecular and involves the nonspecific association between proteins when

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

Box 3. How many copies of a folded protein are unfolded in a cell?

Average folding free energy of a yeast protein:[157] $\langle \Delta G \rangle = G_{folded} - G_{unfolded} = -37 \frac{kJ}{mol}$

Average folding equilibrium: $K = \frac{[folded]}{[unfolded]} = e^{\frac{-\langle \Delta G \rangle}{RT}} = e^{\frac{37{,}000 J}{8.314 \frac{J}{molK} \times 300 K}} = 2.8 \times 10^6$

Average unfolded protein fraction: $f_{unfolded} = \frac{[unfolded]}{[folded]+[unfolded]} = \frac{1}{1+K} = 3.6 \times 10^{-7}$

Of course, these numbers miss more proteins than they describe accurately and must be taken with a grain of salt. Nonetheless, it is important to realize how the number of unfolded proteins depends on the stability of the protein. Therefore, for many proteins unfolding is an overall relatively rare event.

they reach high concentrations. Below, we dive deeper into each of these scenarios.

3.5.1. Unfolding due to Water Stress. For many folded proteins, unfolding is a relatively rare event: roughly ∼1 in 40 million proteins with a stable, native conformation are in an unfolded state at ideal conditions at any given time (see Box 3). With a total of ∼70 million proteins in a yeast cell, that means at any given moment only a very small number of well-folded proteins are actually unfolded. Of course, this is a gross estimate and varies widely depending on protein type, location, and the state of the cell. Nonetheless, aside from nascent chains, unfolding is a relatively rare event in a normal cell's cytoplasm. Should unfolding occur as a result of desiccation? While this certainly occurs in some systems,[150,151] several key observations argue against its universality. First is the observation that all intramolecular protein dynamics tend to slow down considerably as water is removed from the system.[152,153] While this does not affect the thermodynamics of the system, it will most certainly affect the rate at which proteins unfold, potentially bringing it to a near-stop as water is removed from the system. Numerous studies showed little or no effect of osmotic challenges or dehydration on protein tertiary structure.[154−156157] As one example, a recent study looking at the structural changes that occur as a cell encounters a rapid reduction in cellular water due to osmotic stress, the folded state of phophoglycerate kinase (PGK, a ubiquitous glycolytic protein) showed no change in structure, even when the cell lost ∼40% of its initial volume from water efflux.[60] Still, most of these studies are anecdotal and examine the state of a single protein, preventing us from drawing strong conclusions about the vast diversity of proteins within the proteome.

3.5.2. Aggregation Due to Water Stress. Another possibility for protein dysfunction is irreversible aggregation brought about by dehydration (Figure 6). In this process, proteins stick to each other nonspecifically to form an aggregate. Often, these aggregates form irreversibly and, with some exceptions,[158] cannot be untangled, leading to their loss of function. In line with this, a hypothesis floated recently is that the cellular proteome is often on the verge of solubility.[159,160] This means that, on average, proteins inside the cell are just at (or even above) their solubility limit, and any increase in concentration can result in their precipitation. Indeed, protein aggregation has been widely observed both in lysates and *in vivo* for nonresistant organisms,[161] and desiccation resistant proteins mentioned later in this chapter help prevent this phenotype altogether.[30,32,81,162] It is important to note that aggregation dysfunction and unfolding dysfunction need not be mutually exclusive. Aggregation is often tied in with misfolding,[163] and structural characterization of desiccation-induced aggregates has not been well resolved.

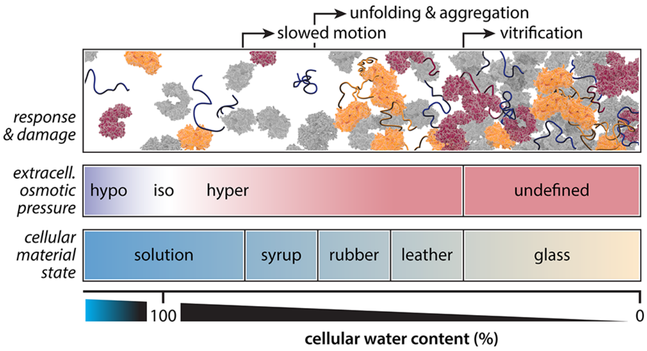

Figure 6. Protein damage under water stress. As osmotic pressure sets in and water leaves the cell, protein motion begins to slow. Both internal and translational movement begins to slow down as the environment becomes more viscous. This is expected to occur in a size-dependent manner, with larger protein complexes being affected earlier at the onset of water stress. At the same time, a lack of water availability disrupts the balance between the cellular environment and protein surfaces. Increasing ionic strength causes some electrostatically driven complexes to dissociate and drives aggregation between proteins with like charges. Changes in water availability may also disrupt the structure of well-folded proteins and lead to un/misfolding. As water continues to leave and the space between proteins is further reduced (see Box 1), aggregation of proteins and vitrification of the once-aqueous environment set in.

Because they are not mutually exclusive, and indeed are often linked together, both aggregation and unfolding likely occur concomitantly in desiccating cells.

4. INTRINSICALLY DISORDERED PROTEINS: A BUFFER AGAINST DESICCATION DAMAGE?

A significant portion of most proteomes does not assume a stable three-dimensional fold and instead exists in an ensemble of rapidly interchangeable conformations. Such intrinsically disordered proteins or protein regions (IDPs and IDRs) are different from well-folded proteins. The lack of a tertiary structure in IDPs is driven by the absence of bulky hydrophobic amino acid residues and an enrichment of charged and polar residues.[164,165] In stark contrast to the hydrophobic core of a well-folded protein, IDP ensembles possess few intramolecular bonds and a high degree of solvent accessible surface area.[166−168] This feature allows for several unique functions, including binding multiple partners with exceptionally high specificity[169−171] and an outsized sensitivity to changing environmental conditions.[60,172,173] Several reviews have been written on IDRs that specialize in protecting the intracellular environment from desiccation.[35,148,149]

4.1. IDPs in a Dehydrating Cellular Environment

Disordered proteins have been shown, both *in vitro* and in live cells, to be highly sensitive to changes in abiotic conditions, including pH, osmotic pressure, and solute composition. Despite not having a fixed three-dimensional structure, IDP ensembles have structural preferences that determine their average shape. Increasing cosolute concentrations, crowding, and ionic strength can all have dramatic effects on these structural preferences for some IDPs.[172−174] This can be seen by the expansion or compaction of the average diameter of the ensemble, measured using Forster resonance energy transfer (FRET) or small-angle X-ray scatter (SAXS) as a change in the radius of gyration (R_g) or the end-to-end distance (R_e).[174−176] It can also be the formation of localized secondary structure, primarily helices, in certain regions of the protein, as measured using circular dichroism or NMR.[177−180]

Why are IDP ensembles so susceptible to the composition of their surrounding solution? Two primary factors work together to enable this: (1) Without an extensive network of intramolecular bonds, IDP ensembles are highly susceptible to changes in their conformation. For example, the addition of even low concentrations of urea (<1 M) cannot change the structure of most folded proteins but can act to dramatically expand disordered sequences.[172] (2) The extended surface area that is present in many disordered proteins exposes them to extensive interactions with their surrounding solutions. Thus, if a solution constituent has strong attractive interactions with polar residues (and repels hydrophobic residues), the regions of the IDP with extensive polar residues will be exposed (and hydrophobic regions buried), in line with the enthalpic effects described in section 2.1.2 (see also Figure 4). In the context of desiccation tolerance, IDP sensitivity to their environment raises an obvious question: why would proteins whose structure is extremely sensitive to changes in their surroundings be the weapon of choice for combating the changing environment in a desiccating cell? It has been suggested by us and others[151,181−183] that this sensitivity is precisely what allows IDPs to adapt efficiently and in real time to buffer out the deleterious physical effects that occur because of water stress.

IDPs are able to adopt preferred conformations as their surrounding environment is subjected to desiccation. This conformational change allows them to modulate their interactions with the environment by exposing and burying specific amino acid residues. For example, the formation of amphipathic helices under desiccation is a prevalent feature of many protective IDRs.[177−179,184] An alternative option is that in rapidly dehydrating systems, disordered proteins can take up more space and replace water as a lubricant, preventing other well-folded proteins from sticking together.[148,149,180] Of note, these different mechanisms are not mutually exclusive, and each IDP may function through a combination of both structural changes and higher-order assembly mechanisms (as we will discuss below), as well as through other undiscovered modes of action.

The precise mechanism of action of how IDPs counteract desiccation and play a role in anhydrobiosis is still poorly understood and the topic of intense research. Regardless of what this is, several families of desiccation related IDPs have been identified. In this context, it is important to mention that the determination of homologous function in IDPs is notoriously difficult due to poor primary sequence conservation.[185] While our ability to identify sequences as disordered is adequate, the ability to infer function from sequence is still a daunting task and almost nonexistent. Nonetheless, for desiccation protectant IDPs, some homology has been found in the form of specific sequence features.[186,187] Below, we cover some IDP families that have been linked with desiccation protection in a range of organisms.

4.2. LEA Proteins

One of the first groups of IDPs associated with DT are the LEA, or late embryogenesis abundant proteins. These are a group of mostly disordered and highly conserved proteins shared among anhydrobiotic organisms. LEA proteins were initially found to accumulate during the acquisition of desiccation tolerance in orthodox seeds, suggesting they play a role in DT.[182,188−190] Later on, these proteins were also implicated in the water stress response in plant vegetative tissues in the context of drought,[191] freeze tolerance,[192,193] osmotic stress,[194,195] and the desiccation of resurrection plants.[196,197] In addition to plants, LEA-like proteins have also been identified in a variety of other organisms, where they promote DT. Examples include bacteria,[87,198] nematodes,[199] chironomids,[200] brine shrimp,[201] and rotifers.[202]

LEA proteins are classified in different groups based on the conservation of motifs in their sequence. However, the different groups do not share sequence similarities apart from their high hydrophilicity, making structural and functional comparisons almost impossible. While multiple mechanisms have been proposed for LEA proteins to explain their function during water stress, for example, as membrane stabilizers,[203,204] metal ion scavengers,[205,206] protein stabilizers,[207−210] and glass stabilizers,[211,212] their actual mode of action is still largely unresolved.[148,204,212]

Despite our lack of knowledge on their molecular mechanism and precise function, LEA proteins have drawn attention from the IDP field owing to their ability to change their structure in response to molecular crowding and water availability changes. This is the case of LEAs from groups 3, 4, and dehydrins (group 2) that take up α-helical folds in solutions containing osmolytes, such as glycerol or ethylene glycol but also in the presence of polymers that mimic molecular crowding, such as PEG and Ficoll, and even upon desiccation.[177,179,213] These observations have generated the hypothesis that LEA proteins could act as environmental sensors that detect changes caused by water stress. This feature was exploited by Cuevas-Velazquez and co-workers who used a LEA4 sequence to design a fluorescent biosensor able to detect changes in the molecular crowding in bacteria, yeast, mammalian, and plant cells.[214] Interestingly, the structural changes that LEA proteins show are not limited to their secondary structure. It has been reported that COR15, a LEA from group 3, responds to changes in osmolarity by forming oligomers.[215] This osmolarity-dependent intermolecular interaction suggest LEAs could be forming clusters to carry out their functions in the interior of the cell.

It has been postulated that some LEA proteins could be acting through phase separation into liquid-like or gel-like membraneless compartments.[216,217] While strong *in vivo* evidence is currently lacking, a few studies suggest that this could be the case. First, the IDR of the LEA-SC protein from the nematode *Steinernema carpocapsa* was able to functionally replace the IDR from a yeast P-body protein that is required for condensation.[218] LEA9, a plant LEA that belongs to the LEA_4 group, was found forming condensates in the cytoplasm of hydrated, but not dry, seeds.[219] Additionally, expression of *Arabidopsis thaliana*'s LEA4−5 in yeast cells resulted in the formation of condensates that were dependent on osmotic stress.[214] While these findings are preliminary, they suggest that some LEA proteins could potentially undergo condensation in a manner that is dependent on the water availability in a cell. Yet, future studies should be aimed at addressing this question in the endogenous contexts and test whether perturbing phase separation has actually any phenotypic consequences to DT\

4.3. CAHS Proteins

Perhaps the most well-known DT organisms are the tardigrades or water bears. These microscopic arthropods most commonly live in tidal marine areas or moss patches, both habitats that are prone to repeated wet−dry cycles. When subjected to gradual drying, tardigrades will take up a so-called *tun* state (from the German word for barrel). In their tun state they accumulate specific biochemical components, which makes tardigrades extremely resilient to virtually every stress, from the vacuum of outer space to high doses of radiation.[220] Tardigrades can spend decades in such states of suspended animation, and promptly resume activity upon hydration. While numerous studies had reported on their phenomenal stress tolerance, the molecular mechanism enabling this remained completely unknown.

Boothby and co-workers set out to investigate the mechanism of extreme DT in the tardigrade *Hypsibius dujardini*.[30] They found that tardigrades express a set of intrinsically disordered proteins, cytoplasmic abundant heat soluble or CAHS proteins, upon drying. CAHS proteins did not resemble the sequence features of LEA proteins and seemed exclusive to tardigrades. RNAi knockdown of several of these proteins reduced survival after desiccation. Moreover, when expressed in bacteria or yeast, certain CAHS proteins would confer increased DT to these organisms, showing that they directly contribute to tardigrade DT.[30] Follow-up work indicated that CAHS proteins undergo reversible gelation when subjected to drying *ex vivo*. Interestingly, mixing desiccation sensitive enzymes with CAHS proteins in the test tube preserved enzyme activity after desiccation−rehydration.[85]

The mechanism of protection for CAHS proteins is the subject of intense studies.[180,221,222] CAHS proteins possess gelation properties which may tie into their ability to vitrify and protect cellular environments. Indeed, recent work showed that CAHS mutants with altered gelation properties differed in their protective capabilities compared to the WT protein.[221] The protective action of such gels could be attributed to the slowed diffusion and water coordination within the gel phase. Even though *in vivo* gelation has not been directly observed for these proteins, differential scanning calorimetry (DSC) showed that microbes expressing CAHS proteins have altered glass transition temperatures. As we have discussed above, when organisms dry out, their intracellular contents vitrify. Because the parameters of such biological glasses (e.g., strength) can be assayed via DSC,[223] these changes in glass transition temperatures indicate that CAHS expression does alter aspects of cytoplasmic vitrification. This effect could be attributed to their gelation propensity, as such network-spanning interactions would be expected to promote glass strength, explaining the increased desiccation tolerance promoted by these sequences.[221]

4.4. FLOE Proteins

Plant seeds are specialized propagating vectors that, in most plant species, reach a quiescent state of DT that allows them to remain viable in harsh conditions for up to thousands of years.[224−226] They do so by accumulating protective molecules (including LEA proteins) and changing the biophysical properties of their cytoplasm from a fluid to a glassy state that stabilizes cellular components and halts metabolism to a near standstill.[227,228] Upon water uptake (imbibition), seeds refluidize their cytoplasm and undergo a series of biochemical events that lead to the resumption of metabolic and enzymatic activities. However, until they make the critical decision of germinating, seeds maintain their DT and can undergo multiple hydration−dehydration cycles while remaining viable.[229−232] Once committed, they can no longer revert to the dry quiescent state.[233] Thus, when poorly timed, seedling establishment will be either arduous or unsustainable.[234] This presents an exceptional challenge to the dormant embryo: it needs to predict the future availability of water and act accordingly, germinate when plenty of water is around for the seedling to survive and stay dormant when conditions are limiting. Despite the ecological and agronomical importance of germination, it has until recently remained largely elusive how seeds pull off this extraordinary feat.

In recent work by us and others, we hypothesized that disordered proteins could play a role in sensing the transition from the dry to wet state. While LEA proteins have been abundantly implicated in seeds (see above), we specifically focused our attention on another lesser studied class of IDPs. So-called prion-like proteins are prime sensor candidates. These proteins can undergo reversible phase transitions, and work in yeast has shown that such transitions can allow populations of cells to sense and respond to a change in the environment.[235,236] We identified a previously uncharacterized prion-like protein that was expressed in a seed-specific manner in *Arabidopsis*. This protein, which we named FLOE1, could undergo a surprising hydration-dependent phase transition: in the desiccated embryo, FLOE1 is diffusely localized to the cytoplasm, but it spontaneously forms condensates upon hydration of the embryo.[237] Importantly, this process is fully reversible, as seeds that are redried after imbibition do not exhibit any condensates unless they are exposed to water once again. Another striking feature of these FLOE1 condensates was the exquisite sensitivity of their formation to water potential: the lower the water potential, the less cells in the embryo present with condensates and *vice versa*.[237]

While the FLOE1 protein seems to sense the water potential, it was still unclear if this function would affect germination. Testing germination of FLOE1 knockout and overexpression lines showed that the protein acts as a dose-dependent inhibitor on germination under water stress. In other words, seeds lacking FLOE1 will germinate prematurely at water potentials that may not suffice to support the seedling, while overexpression lines require higher water potentials for germination. Nonetheless, the question remained whether this functional effect relates to its phase separating properties. By generating a mutant form of FLOE1 that lost its hydration dependency (i.e., was always in a condensed state), we found that these lines had aberrant germination responses. Seeds with constitutive FLOE1 condensates behaved as knockout seeds but with an even stronger premature germination response in conditions of low water potential. These findings demonstrated

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

that the reversibility of FLOE1 phase separation was absolutely critical for its function.

The discovery of the function of FLOE1 brought up an interesting question: because FLOE1 phase separation can be tuned by its expression level and sequence, do plants use these parameters as tuning knobs to fine-tune their seeds' germination properties to the local environment? By analyzing (isoform) expression data from hundreds of *A. thaliana* ecotypes, we indeed found that FLOE1 expression is correlated with the local climate *and* germination phenotypes across wild populations.[237] Lastly, the FLOE gene family is conserved across the green plant lineage and the number of homologues per species has increased over the course of plant evolution, from one homologue in unicellular algae to up to nine homologues in certain angiosperms, highlighting the wealth of FLOE1 variation plants can make use of. Interestingly, a recent study revealed that FLOE2, a homologue widely expressed across *Arabidopsis* tissues, is implicated in the drought tolerance of leaves.[238] Both these findings suggest that the FLOE family could play broad roles in water stress and DT across the green plant lineage, with potential applications for crop design and agriculture.

While interesting, we would like to note that many questions regarding the function of FLOE1 remain unanswered. First, its precise molecular function and mechanism of action is still not completely resolved. Because FLOE1 is dispersed in the dry state of the embryo, some of these analyses represent technical challenges. Biochemistry typically requires water, and new efforts should be aimed at repurposing technology from the material science field to investigate dry biological matter. Second, we have currently only assayed FLOE1-related phenotypes in *Arabidopsis*, and replication in other organisms will be required to test whether this may be general function of FLOE genes across the plant lineage.

More than likely, many other protein families that can protect from desiccation exist. Furthermore, even the breakdown of the families is biased by our thinking about protein homology, which is firmly rooted in structured proteins and their evolution. The molecular rules that underlie the protective ability of IDRs, as well as the mechanisms by which they function, remain to be discovered.

5. BIOMOLECULAR CONDENSATION AS AN EMERGING THEME IN DT

The hydration-dependent phase separation of FLOE proteins (and potentially certain LEAs), together with the gelation behavior of CAHS proteins, suggests that protein condensation and other phase transitions could be implicated in the molecular mode of action of these desiccation protectants. For DT organisms from across the Tree of Life, we do see very similar disordered proteins being expressed during time of water stress. While IDPs can have many modes of action (see above), the recent appreciation of their overrepresentation in biomolecular condensates prompts us to ask whether changes in biomolecular condensation could be a more widespread phenomenon when water gets limiting?

From countless *in vitro* experiments, we indeed know that protein phase separation is exquisitely sensitive to the (bio)chemical conditions and critically dependent on the concentration of the scaffold proteins themselves, as well as ionic species and metabolites. Therefore, the effects of desiccation on different proteins and biomolecular condensates may be complex and hard to predict (Figure 7).[239] When we consider a simple two-component polymer−solvent system, removing solvent from the system will drive up the concentration of the polymer, eventually pushing it over its saturation concentration leading to a demixing of the system. While in certain scenarios proteins may follow such a pattern in a drying cell, it is naive to assume that this would be the only possible outcome. We have discussed in section 3 how the concentration of cosolutes may change by orders of magnitude and how the metabolite pool can drastically change during priming. Also, specific entropic/enthalpic and kinetic effects will differentially impact the behavior of different proteins. FLOE1 is a perfect example of such a potential contradictory effect. *In vitro*, its phase separation is heavily concentration-dependent, and a similar dependency is observed when expressed in human cells as "living test tubes". Yet, at endogenous expression levels in plant seeds, FLOE1 droplets *dissolve* when seeds dry out despite the obvious rise in its intracellular concentration due to the loss of water.[237] This example shows that during drying, the sum of several (potentially opposing) effects should always be considered. It further suggests that the extent of dehydration will be important in determining the downstream effects on condensation phenomena, as certain promoting/inhibiting mechanism may change in relative importance at certain levels of water loss. Additionally, once the water potential stabilizes, adaptation can occur as we have seen for priming.

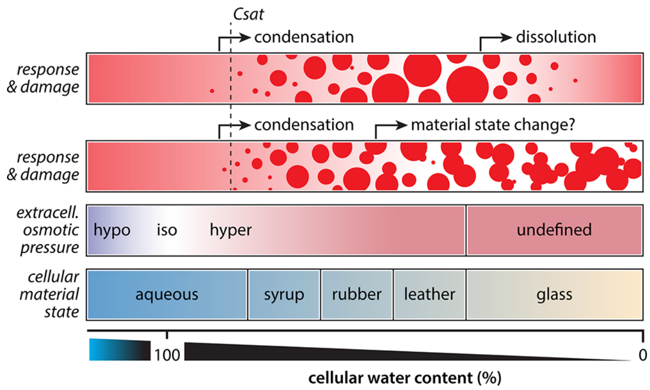

Figure 7. Condensates under water stress. As the water content in the cell is lost, the properties of condensates can be altered in several different ways. As osmotic pressure sets in, water leaves the cell causing the intracellular concentration to increase, which can trigger condensation when the critical saturation concentration (C_{sat}) is reached. As more water leaves the cell, different scenarios can be at play. Condensates likely can change material state, taking up more solid-like features (depicted as arrested fusion events) as they get depleted from their solvent. As we have seen for FLOE1, condensates can also dissolve during vitrification, yet the molecular mechanism driving this is unresolved.

While biomolecular condensation has been investigated over a range of stress conditions (e.g., heat shock, arsenite exposure), the implications for water stress and DT remain vastly understudied. Yet, given the high sensitivity of these assemblies and the involved proteins to changes in their solvation, and the handful of examples seen above, we are convinced that many exciting discoveries are waiting to happen. Below, we will discuss the current evidence that links biomolecular condensates to water loss and the adaptive response to it.

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

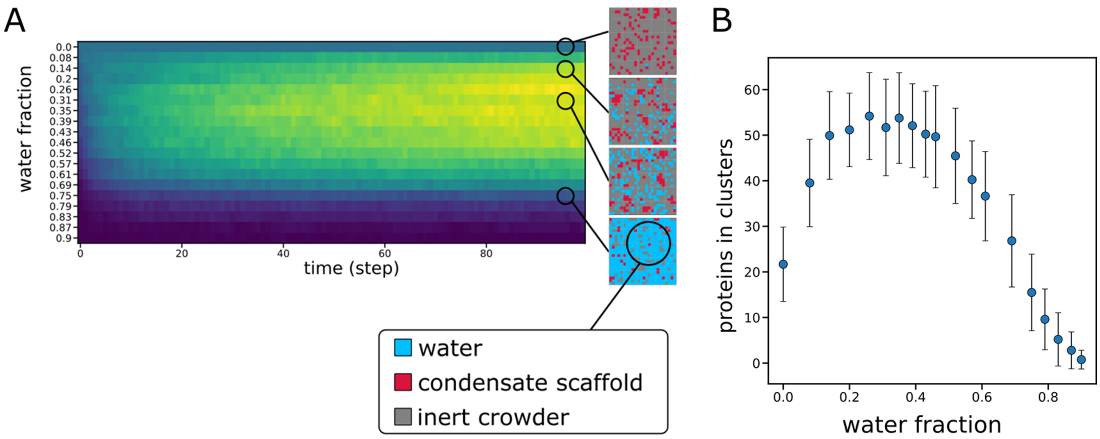

Figure 8. Simulations of liquid−liquid phase separation in dehydrating cells. (A) A time series for condensate formation simulations with different water contents. All simulations based on the on-grid Monte Carlo simulation by Watanabe et al.[240] Each row represents a simulation grid with a fixed number of condensate-forming scaffold proteins (red), inert crowders that do not get included in condensates (gray) and water (blue). The progression of each simulation is shown in time steps on the x axis. For all grids, the number of scaffold and inert proteins is held constant, and water content is increased by changing the area of the grid. The colors represent the number of proteins in clusters larger than 6 proteins (dark blue to yellow indicating increasing numbers). Maps on the right hand side show a snapshot from the simulation. Note that for high water fractions, this is only part of the simulation grid. All data is obtained by averaging over 50 independent trajectories. (B) A cut through the heatmap in (A) at t = 90 steps shows the number of proteins in clusters as a function of fraction of water grid spaces. Circles and error bars are averages and standard deviations of all 50 simulations.

5.1. Intermolecular Interactions Modulate Condensation during Dehydration

We have discussed how IDPs and biomolecular condensates are exquisitely sensitive to changes in their physical-chemical environment, including water content. But, during dehydration, several mechanisms that influence these proteins' behavior are at play, with currently hard to predict outcomes. Specifically, the interplay between elevated solute concentrations and reduced diffusion can play out in complex ways that strongly depend on the rate of desiccation, the identity of the solutes involved, and the cosolute environment as the cell dehydrates.

To visualize how the competing factors of increasing concentrations and reduced diffusion may affect condensation, we used the Monte Carlo simulation program developed by Watanabe and co-workers[240] to simulate a "dehydrating" cell (Figure 8). These simulations consist of a 2D grid occupied by condensate-forming proteins, inert "observer" proteins ("obstacles" in the original publication), and solvent spaces. To simulate desiccation, we held the number of condensate-forming proteins and inert crowders fixed and reduced the number of "water" spaces by compacting the simulation grid, going from an 80 × 80 grid (where 90% of the grid was "water") to 25 × 25 (where there were no "water" tiles at all).

At high water levels, condensates do not form because the critical concentration for condensate formation, C_{sat}, is not reached at this dilute state. As the water content drops and condensate-forming tile concentration increases, condensates begin to emerge. A peak at the rate of formation, as well as the extent of condensates proteins in cluster, is reached at around 30% "water". As the water content continues to drop, a visible slowing down of condensate formation appears, where finally at 0% "water" the system is jammed and can no longer form condensates (beyond those that are already formed when the grid was initialized).

Clearly, the outcome of these competing effects depends on multiple parameters, including the C_{sat} of the scaffold proteins and their interactions with so-called "inert" proteins and cosolutes, as well as the specific changes that occur inside the cell during drying. It is clear, however, that outcomes can be varied, and especially in realistic cases where desiccation occurs gradually over hours or days, there could be unique advantages for sequestering the biomolecular machinery of the cell inside condensates.

5.2. Surveying Condensate-Forming Proteins That Respond to Desiccation

The study of how desiccation affects biomolecular condensates is still in its infancy. While it is very likely that the extreme changes in the physicochemical properties that biological systems experience during desiccation will affect virtually any condensate, the functional implications of this remain to be explored. Are such observed changes detrimental, or do they actually contribute to DT and act to protect cells? Below, we will discuss the limited evidence we could find of reported changes to condensates in desiccated systems. By no means though do we think that the sparse reports in this area are in line with the actual biology. On the contrary, this summary should act as a stimulant for others to explore how desiccation may affect other condensates.

While still pretty unexplored territory in desiccation, condensates have been more extensively studied in osmotic stress. In this context, it is important to mention the work of the Walter group, who took a systematic look at the ability of different condensate scaffold proteins to respond to hyperosmotic stress.[241] The group reports that specifically multivalent proteins can reversibly form condensates in response to hyperosmotic stress. But again, many questions remain as to the contents of such osmotic stress-driven condensates and its physiological relevance to counteract water stress. Below we survey several examples of known condensates and their reported connection to changes in cellular water levels and highlight how these changes may contribute to recovery from osmotic stress or desiccation.

5.2.1. Nucleoli. The nucleolus represents the largest and most well-known biomolecular condensate. This compartment mainly functions as an assembly line for the manufacturing of ribosomal subunits. Ribosomal RNA (rRNA) is transcribed in the center of the nucleolus and will subsequently undergo a range of processing steps when it travels toward the nucleolar periphery though the different layers (aka centers) of the nucleolus. Concomitant with the efflux of rRNA, there is an influx of ribosomal proteins, which will latch onto the rRNA to form the ribosomal subunits.[242] This mechanism is responsible for the production of the millions of ribosomes that are present in a cell at a dazzling rate of 4000 ribosomes per minute.[243] Because ribosome production is one of the main energy expenditures of a cell under homeostasis, this process is tightly regulated by environmental cues.[244] A variety of stressors will lead to the cessation of rRNA transcription, as the cell no longer needs the production of new ribosomes but rather aims at directing energy at coping with and recovering from the insult. The loss of rRNA transcription results in gross morphological rearrangements of the nucleolar multilayered topology and altered partitioning of key nucleolar factors.[244] In mammalian cells subjected to osmotic stress, there is a largely identical remodeling of and reduction in the nucleolar volume,[245,246] differential partitioning of nucleolar factors,[247] nucleolar stress cap formation, and the accumulation of DNA damage through R-loop stabilization.[248] Similar responses have been described for other eukaryotes, such as yeast and *Arabidopsis*.[249,250] These observations suggest that the nucleolus is a conserved stress signaling hub in times of water limitation, and highlight the potentially complex contributions from abiotic (e.g., changing component concentration) and biological (i.e., transcriptional shut-down) events to its remodeling. Lastly, water stress-induced compositional changes—for example, the departitioning from an epigenetic silencer from the nucleolar matrix,[249] may indicate unappreciated ways of how the nucleolus can regulate adaptive water stress responses.

Another set of intriguing observations comes from many decades-old studies that report careful microscopic observations of germinating plant seeds.[251−253] The nucleolus undergoes gross size changes and vacuolization events over the course of both seed maturation (i.e., dehydration) and germination (i.e., rehydration). Despite many years since these initial discoveries, what all of these events mean for actual nucleolar biology and function remains yet to be shown.

5.2.2. P-Bodies. Processing bodies or P-bodies, are cytoplasmic condensates that store translationally repressed mRNAs and mRNA decay machinery.[254] While they were initially suggested as the sites of active mRNA decay, their presence is not required for RNA degradation[255] and mRNAs can shuttle out of P-bodies to resume translation.[256] Moreover, a recent study shows that the absence of P-bodies actually results in higher mRNA degradation rates in yeast,[257] indicating that degradation factors may be inactivated by condensation. This idea is in line with *in vitro* reconstitution assays that show that mRNA is protected from RNA cleavage and decapping within the condensates.[258] These studies suggest that P-bodies may serve as temporary storage sites of mRNA that do not require immediate translation in a specific moment and therefore make them key sites for rewiring gene expression under times of stress. P-bodies do enrich in specific mRNAs during stress, which may buffer detrimental and promote adaptive changes in gene expression.[259,260] They also grow in size and number under osmotic stress in *C. elegans*,[261] yeast,[262,263] and human cells.[241] It is believed that this increased condensation simply follows the increased intracellular concentration of their scaffold proteins upon water loss.[241] While the exact mechanism by which these condensates orchestrate mRNA translation remains up for debate, work in yeast has shown that their precise temporal regulation is key to a cell's recovery from osmotic stress.[264] This provides strong evidence that P-bodies are not just water potential sensors but may be directly involved in dehydration adaptation. Future studies could test this by designing P-bodies with alternate water responses and evaluate their effect on fitness under water stress.

5.2.3. ASK3 and WNK1 Kinases. Apoptosis signal-regulating kinase 3, ASK3, is a kinase implicated in mammalian osmoregulation via a signaling cascade that controls the activity of ion channels.[265] Upon hypotonic stress, cells swell and ASK3 gets phosphorylated, driving its activation and hereby inhibiting certain downstream ion channel regulators. The opposite is true in times of hypertonic stress, where the decrease in cell volume coincides with ASK3 its dephosphorylation and inactivation, resulting in the activation of specific ion channels for the absorption of salt ions to balance the osmotic pressure. Yet, it remained unclear how exactly ASK3 was inactivated due to its phosphorylation. Recent work showed that ASK3 forms condensates under hypertonic conditions and that its condensation is required for its proper inactivation.[240] Because ASK3 knockout mice present with hypertension due to failure of kidney osmoregulation,[265] it provides an excellent case study to interrogate the biological function of dehydration-dependent condensation in an *in vivo* animal model.

Besides ASK3, also with-no-lysine kinase 1, WNK1, was recently found to condense upon hyperosmotic stress.[266] WNK1 was already known to be involved in regulating ion channel activity, but the high ion concentrations that accumulate upon hypertonic shock should potently prevent WNK1 activity. It has now been suggested that WNK1 condensates create a biochemical environment that potentially shields it from its ion inhibitors and promotes its activity, allowing cells to restore more quickly from their volume reduction.

5.2.4. YAP. Yes-associated protein (YAP) is a transcriptional coactivator that is regulated by the Hippo pathway. This pathway responds to mechanochemically induced changes in tissue morphology and osmolarity.[267−269] Active YAP localizes to the nucleus and turns on TEA domain family (TEAD) transcription factors that promote cell proliferation and differentiation.[270−272] Despite the significance of YAP, not much was mechanistically known regarding its nuclear translocation and ability to alter gene expression patterns. Osmotic stress can alter the tension on the nuclear membrane, resulting in an opening of the nuclear pore complexes and YAPs entry from the cytoplasm.[273] Once in the nucleus, YAP condenses and remodels euchromatin, recruiting transcriptional machinery in the process and driving gene expression.[274] While this mechanism can be used by cells to respond to sudden changes in water potential, evidence suggests it could also be involved in long-term adaptation to stress and tissue function. The mammalian kidney is characterized by an isotonic cortex and hyperosmotically stressed medulla. Compellingly, YAP is diffusely localized in cortex cells, while presenting with nuclear puncta in the medulla. This finding indicates that tissue-specific osmolarity-dependent condensation of a protein may be critically important for kidney functioning. It also suggests that the kidney could be an ideal

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

place to look for other water potential dependent condensation events in the mammalian context. Lastly, given that YAP is commonly overexpressed in cancer,[275] could its osmo-sensing function be implicated in tumor resilience? When tumors grow, they become constrained by the surrounding extracellular matrix, leading to compressive stress.[276] Intriguingly, tumor cells adapt to this by lowering their intracellular tonicity. As predicted from observations made in the kidney, YAP has been observed to form nuclear condensates in breast tumors compared to healthy breast tissue.[275] Thus, YAP's osmo-sensing activity through condensation seems important for both its physiological and pathological function. Additionally, finding ways to selectively and site-specifically drug YAPs condensation could provide a promising antitumor strategy as it would cripple cancer cells in their long-term adaptation to the stress in the tumor microenvironment.

5.2.5. Nuclear Speckles. Nuclear speckles are RNA-protein condensates that are found in the interchromatin regions of the nucleus.[277] Just as for the nucleolus, it was recently shown that these condensates have a concentric layered topology,[278] but the biological importance of this architecture remains unknown. Functional studies have pointed at multiple roles for nuclear speckles, including transcription regulation, cotranscriptional splicing, mRNA export, and genome organization.[279] Given their central role in gene expression, it may come as no surprise that nuclear speckles could be key sites of stress sensing and response. Indeed, several nuclear speckle-associated splicing factors have been directly implicated in the response to drought and salt stress in plants.[280] Additionally, in plant cells,[281] rat neurons,[282] and human cells,[283−285] nuclear speckles undergo morphological rearrangements and compositional changes under water stress. A prime example of such a water stress sensor is HIN1.[281] Upon drought stress in *A. thaliana*, HIN1 partitions into nuclear speckles, which coincides with broad changes in intron retention. Overexpression of HIN1 in unstressed plants partially mimicked these stress-induced splicing changes, and the implicated transcripts were enriched for HIN1 RNA binding motifs, highlighting its direct involvement. Moreover, overexpression of HIN1 protected seedlings against water stress, while HIN1 knockout plants were sensitized. This example shows that stress-induced changes in nuclear speckle composition can alter organismal fitness by regulating alternative splicing of stress response transcripts. These findings provide a way of how one could engineer entire gene networks to generate drought-resistant agricultural crops in light of climate change.

Lastly, in systems that undergo full desiccation, such as fern microspores, nuclear speckles have been shown to undergo dramatic remodeling events as they coalesce into one large assembly. While still incompletely resolved, it has been suggested that this event is required for asymmetric inheritance of key RNA species during spermatogenesis upon rehydration of the microspores.[286]

5.2.6. Clastosomes. A lot of attention has been given to disordered and RNA-binding proteins, even though condensation is by no means limited to them. Clastosomes or proteasome granules provide perhaps the most compelling example of such "unconventional" condensates. The proteasome is a multisubunit degradation machine consisting of folded subunits in a specific stoichiometry. Proteasomes are normally present diffusely in both nucleus and cytoplasm, yet, upon osmotic stress they condense into nuclear foci, called clastosomes. This behavior has been observed in cells *in vitro*[246] and *in vivo*.[287] These foci are also enriched for ubiquitinated substrates, and treating cells with proteasome inhibitors influences clastosome formation and disassembly,[246,287] suggesting that they are sites of protein degradation. Clastosomes recruit ribosomal proteins as substrates for degradation.[246] As we have seen above, osmotic stress shuts down the nucleolar ribosome assembly line, hence, the need for their efficient degradation when ribosomal proteins are in excess. Studies in *C. elegans* have shown that osmotic stress induces the ubiquitination of a wide array of proteins, beyond ribosomal ones, and that their degradation is key to recovery from stress.[288,289] Thus, the stress-induced formation of degradation factories through biomolecular condensation constitutes an elegant adaptive mechanism to prevent protein aggregation and prime the cell for stress recovery.

5.2.7. Chromatin. Eukaryotes organize their DNA by wrapping it around nucleosomes, consisting of octameric assemblies of histones. While traditionally not considered a condensate, recent studies have found that histones and related proteins can undergo DNA-mediated condensation *in vitro*.[290−292] These observations have suggested that chromatin, or histone-bound DNA, may share some behaviors with more classical condensates in cells.[293] This especially seems true for heterochromatin, the inactive and compacted form of chromatin.[294] Given its containment within the nuclear envelope, the dramatic size reduction of the nucleus during osmotic stress is expected to drastically compress and remodel the chromatin. Electron microscopy studies in both yeast, rat, and human cells have exactly shown this to be the case.[250,282,285] While this compacted chromatin has the same density as heterochromatin, its mere compaction is not sufficient to drive the recruitment of the classic heterochromatin proteins or histone marks.[295] Hence, this compaction seems completely reversible upon relief from osmotic stress.[295]

Although chromatin is reversibly impacted by osmotic stress when cells are in interphase, in mitotic cells this picture is vastly different. For mitosis to occur, cells must condense their chromosomes, which can subsequently align on the spindle and be distributed among daughter cells. One issue is that these mitotic chromosomes are "sticky" and hence can easily congeal together. To circumvent this, human mitotic cells express Ki67, which is a long disordered cationic protein that coats the chromosome surface. By forming a positive charged layer around each chromosome, Ki67 effectively acts as a surfactant preventing different chromosomes from sticking together.[296] Upon osmotic stress, however, Ki67 is no longer able to form its protective coating, resulting in the formation of one large agglomeration of condensed chromatin interfering with proper mitotic progression.[297] Although it is not clear how well-conserved this mechanism is across eukaryotes, osmotic stress is known to induce mitotic defects in other organisms, such as plants.[298]

5.2.8. Other Osmotic Stress-Induced Condensates. Both the cytoplasm and nucleoplasm of a stressed cell are characterized by stress-dependent condensates, osmotic stress being no exception to this rule. Stress granules are cytoplasmic ribonucleoprotein assemblies that specifically form under a variety of environmental and biological stresses in eukaryote cells.[299] While stress granules typically form through the condensation of G3BP RNA-binding proteins and free cytoplasmic mRNA upon polysome disassembly, remarkably,

https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

osmotic stress granules do not require any of these two events to occur.[300,301] A recent study showed that sorbitol treatment triggers the rapid clustering of IGF2BP RNA-binding proteins in human cells.[302] Upon maturation, these clusters coalesce and recruit classic stress granule markers such as G3BPs. While the resulting granules look largely similar, their biogenesis clearly follows a different route. So why do cells form stress granules? For a long time it was believed that these granules were responsible for the protection of mRNA after stress-induced polysome disassembly, which turned out to not be the case.[303] Additionally, these assemblies were suggested to drive the formation of protein aggregates typically found in neurodegenerative diseases.[304,305] More recent evidence suggests that they actually could provide an initial protective response against aggregation by keeping aggregation-prone proteins in an immobilized and protected RNA-bound state.[158,306−308] How such a function would aid in recovery from osmotic stress remains to be tested.

Just like the formation of cytoplasmic stress granules, a variety of stressors will drive formation of nuclear stress bodies.[309] Also called SatIII bodies, these condensates depend on the stress-induced transcription of specific noncoding RNAs, called satellite III repeats. HSF1 is a eukaryote-conserved and crucial transcriptional regulator that orchestrates the cellular transcriptional response to several stressors,[310] including the expression of SatIII repeats.[311] Yet, under osmotic stress HSF1 is surprisingly dispensable for SatIII expression, and this role is taken up by NFAT5. This transcription factor will undergo osmotic stress-dependent nuclear translocation and localizes to the SatIII bodies.[311] Of note, reminiscent of what we had seen for YAP, NAFT5 translocates from the cytoplasm to the nucleus under conditions of hyperosmotic stress.[312] Concordant with this, knockout mice present with gross atrophy of the hyperosmotic medulla of the kidney, yet retain a normal (isotonic) cortex.[313]

Besides the examples highlighted above, several other proteins have been found to undergo condensation during osmotic stress in mammalian,[314−316] yeast,[317,318] fly,[319] and plant[320−322] models.

6. CONCLUSIONS AND OUTLOOK

This review aims to provide an overview of the overlap between our understanding of water stress and condensates but will hopefully also inspire the reader to think deeper about the chemical, physical, and biological mechanisms at play during desiccation and how we can study them. As life evolved in or around water, its presence is considered an absolute necessity for cells to exist. Despite this, cells routinely encounter water-limiting conditions or even pursue desiccation purposefully. While water stress and anhydrobiosis have been studied for many years, due to the difficulty of working with dry material, a lot of the accrued work has been observational in nature. In the past decade, more studies have taken a deep dive into the potential ways in which cells adapt to drying. IDPs fill the role of desiccation protectants in organisms across all kingdoms of life. With their sensitivity to the environment and their extended surface area, IDPs can be ideal sensors of water content and offer an adaptable response to water loss. Given that these dynamic proteins are also enriched in the newly appreciated dynamic biomolecular condensates, we decided to explore in this review the potential contribution of protein phase separation to desiccation tolerance. A major issue standing in the way of understanding desiccation tolerance is that biological systems in a vitrified, desiccated state cannot be studied by standard biochemical and molecular biology approaches. Indeed, most assays require that organisms and proteins be hydrated following desiccation to assess protective ability. Even basic thermodynamic treatment of biological systems usually relies on the presence of water as an abundant solvent. Yet, tools exist in other fields to study dry matter (e.g., material science), and these are increasingly applied to biological systems as well. Almost a hundred years ago, Kater et al.[253] provided intricately detailed observations of nucleoli remodeling during the desiccation and germination of beans. A hundred years later, most desiccation discoveries hovered over understanding the molecular, genetic, and metabolomic responses that lead to DT. Therefore, the field of desiccation biology is still holding many unresolved questions that require the joint efforts of biophysicists, biochemists, and cell biologists to shed light on exciting discoveries that are waiting to happen. Our hope is that as understanding desiccation becomes increasingly important, new tools will be developed and new insight will be gained, not only into when and where protection is provided, but also into the underlying mechanism of how protective mechanisms work.

Climate change is expected to drastically change weather patterns and subject a range of ecosystems to arid conditions. Figuring out how desiccation affects biological systems, and how such systems have evolved to adapt and survive it, is imperative. The use of condensates is highlighted here as a mechanism that biological systems may have evolved to counteract dehydration and desiccation. In the coming years, we expect our knowledge of how condensate-based protection works will expand. Being able to design de novo sequences that imbue organisms with desiccation protection could have a massive impact on our ability to deal with climate change, both to conserve biodiversity and prevent agricultural collapse. Additionally, a limiting factor in the rapid and equitable global distribution of crucial medicines is the need for a continuous cold chain. Desiccation is a viable strategy for long-term storage of biologicals (e.g., vaccines) and biofluids (e.g., blood) at ambient temperatures. Understanding how to facilitate this through desiccated biomolecules could revolutionize medical access. Given that loss of proteostasis and defects in IDPs and condensates are a recurring hallmark of several age-related degenerative conditions, elucidating how other organisms maintain similarly aggregation-prone proteins in a stable state during stress may even inspire new ways of how we think about drugging these types of proteins in disease. Countless organisms have evolved the ability to survive in the face of one of the most existential threats to cellular life: the lack of water. We are convinced that understanding how this molecular superpower works will bring forth novel technological applications to tackle outstanding challenges in the 21st century.

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https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035

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Notes

The authors declare the following competing financial interest(s): Y.D. and S.B. are inventors on a patent application related to work covered in this manuscript.

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Steven Boeynaems performed his B.Sc. and M.Sc. studies in Bioengineering before receiving his Ph.D. in Biomedical Sciences in 2017 at KU Leuven and the Flemish Institute for Biotechnology (VIB) in Belgium. He joined Stanford University as an EMBO Long-Term Fellow for his postdoctoral work. In 2022, Steven became an Assistant Professor at the Department of Molecular and Human Genetics at Baylor College of Medicine and Investigator at the Jan and Dan Duncan Neurological Research Institute at the Texas Childrens' Hospital in Houston. He is a CPRIT scholar, and a member of the Baylor Therapeutic Innovation Center, Dan L. Duncan Comprehensive Cancer Center, and the Center for Alzheimer's and Neurodegenerative Disease. Work in the Boeynaems lab is focused on understanding how biomolecular condensates encode (dys)function in biology and disease, with a focus on their involvement in stress responses, aging and quiescence.

ACKNOWLEDGMENTS

We thank all members of the Intrinsically Disordered Protein Scientific Interest Group (IDPSIG), IDPseminars, and the Water and Life Interface Institute (WALII) for helpful discussions and suggestions. This work was funded in part by WALII, supported by NSF DBI grant no. 2213983 to S.S. and S.B. S.B. acknowledges funding from CPRIT (RR220094). S.S. and E.F. were funded by NIH NIGMS grant R35GM137926 to S.S. S.R. was funded by NSF IntBIO grant 2128067 to S.S.

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https://doi.org/10.1021/acs.chemrev.2c00659
Chem. Rev. 2023, 123, 9010−9035