Does water stress promote the proteome-wide adjustment of intrinsically disordered proteins in plants?

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Received: 27 November 2017 / Revised: 8 May 2018 / Accepted: 21 May 2018 / Published online: 2 June 2018
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Abstract

Plant response to water stress involves the activation of mechanisms expected to help them cope with water scarcity. Among these mechanisms, proteome-wide adjustment is well known. This includes actions to save energy, protect cellular and molecular components, and maintain vital functions of the cell. Intrinsically disordered proteins, which are proteins without a rigid three-dimensional structure, are seen as emerging multifunctional cellular components of proteomes. They are highly abundant in eukaryotic proteomes, and numerous functions for these proteins have been proposed. Here, we discuss several reasons why the collection of intrinsically disordered proteins in a proteome (disordome) could be subjected to an active regulation during conditions of water scarcity in plants. We also discuss the potential misinterpretations of disordome content estimations made so far due to bias-prone data and the need for reliable analysis based on experimental data in order to acknowledge the plasticity nature of the disordome.

Keywords Water stress · Disordome · Intrinsically disordered proteins/regions

Introduction

The last two decades have witnessed the recognition of the intrinsic disorder concept, which started to challenge the classical view of protein structure/function relationship (Charon et al. 2016). Now, based on a wealth of experimental and bioinformatic analyses, there is wide data about segments and even whole proteins lacking a rigid 3D structure in their native form, but that are fully functional and can play important biological roles (Uversky and Dunker 2010; Stender et al. 2015). These proteins/regions are referred to as “Intrinsically Disordered Proteins” (IDPs) when comprising an entire protein and “Intrinsically Disordered Regions” (IDRs) when they are part of larger structured proteins (Uversky and Dunker 2010). In general, these proteins display low sequence complexity, contain few hydrophobic amino-acids, and exhibit large net charges at neutral pH, as a result of harboring plenty of non-interacting charged groups (Uversky 2002). This gives rise to polypeptide chains

which poses high intramolecular flexibility and slight to missing three-dimensional structure (Kovacs et al. 2008; Uversky and Dunker 2010; Mittal et al. 2013). These features impair to IDPs with their unusually high-temperature stability and solubility under low pH conditions (Cortese et al. 2005; Galea et al. 2006; Zamora-Briseño and de Jiménez 2016). Structural protein disorder is a common physicochemical attribute shared by different protein families, and the collection of IDPs/IDRs of a given organism in a specific condition can be collectively referred as disordome.

Disordome is an ubiquitous feature present in all domains of life, and its content increases from bacteria and archaeobacteria to fungi and eukaryotic organisms, suggesting its value in evolution (Xue et al. 2012; Lobanov and Galzitskaya 2015; Liu et al. 2017). Proteins that belong to the disordome take part in the regulation of important cellular processes, such as transcription, translation, signal transduction, and cell cycle.

It is estimated that around 30 to 40% of eukaryotic proteomes are mostly disordered, and between 82 and 94% of transcription factors have IDRs (Dunker et al., 2001; Tompa 2002; Oldfield et al. 2005; Liu et al. 2006; Yoon et al. 2012; Xue et al. 2012). Several types of post-translational modification sites are disproportionately situated in IDPs/

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IDRs. Around 70% of eukaryotic signaling proteins are predicted to contain IDRs. Both features affect key regulatory processes such as cellular localization, binding interactions, protein stability, and turnover (Iakoucheva et al. 2002; Iakoucheva et al. 2004; Seet et al. 2006). Additionally, certain IDPs can adopt different structures under different conditions or upon contact with partners, enabling versatile contacts with various targets through fast, transient, and weak interactions (Tomba 2002; Gallagher et al. 2006). This enables the re-use of the same protein in different biological processes, gaining dynamic information with no additional energy investment.

It should be remarked that the evidence of IDRs/IDPs functions has been obtained primarily from other models than plants; much less is known about IDPs in plants, even though they contain more IDPs related to environmental adaptation (Tomba and Kovacs, 2010; Sun et al. 2011; Pazos et al. 2013; Pietroseoli et al. 2013; Liu et al. 2017). This consideration can be very important in plants, because they cannot escape from stressful environmental conditions that can compromise their survival. However, there is very little information about the changes in the responsive disordome of plants subjected to environmental stresses. Here, we emphasize the importance and potential involvement of the disordome in response to water stress in plants.

IDPs and plant water stress: a suggestive relationship

The sessile nature of plants has led to the selection of a variety of strategies to confront environmental challenges throughout their life cycles. Water stress is the most important factor which negatively affects plant survival (Andjelkovic and Thompson 2006). In addition, plants must defend themselves against damaging processes associated with water stress, including oxidative stress, membrane destabilization, and loss of macromolecular integrity (Illing et al. 2005).

Loss of macromolecular integrity is remarkably important for proteins, since water is essential for maintaining the structure of many proteins, and its loss can provoke their unfolding and aggregation (Chakrabortee et al. 2007). Loss of water means that a large number of macromolecules must be present in a more compacted cellular space than usual. In some cases, an increase in macromolecular density can be dramatically adjusted, and several mechanisms must be activated to maintain the cell viability when this occurs. This situation can be exemplified by orthodox seeds in dry state or in desiccated tissues of resurrection plants. In such cases, the damage associated with cytoplasmic compaction results in membrane fusion, and protein and membrane denaturation is believed to be prevented by the replacement of water with solutes capable of substituting the hydrogen bonds lost because of dehydration (Farrant and More 2011). One mechanism consists on

accumulating solutes that promote the recovery of cellular components, via water replacement, which contributes to stabilize critical cell components and to increase cytoplasm viscosity (Leprince et al. 2017; Costa et al. 2017). These solutes may include compatible osmolites such as proline, distinct sugars (e.g., sucrose, galactose, arabinose, trehalose, sorbitol/galactinol, gluconate 6-phosphate, and glycylglycyl sucrose, polyols, trehalose), glycine betaine, alanine betaine, proline betaine and pipecolate betaine, organic acids, nitrogen-rich amino acids, and shikimate-derived metabolites (Rhodes and Hanson 1993; Fait et al. 2006). Other strategies comprise also the induction of antioxidant compounds, detoxifying enzymes and chaperones as well as protective proteins such as superoxide dismutases, catalases, glutathione- and ascorbate, peroxidases, peroxiredoxins, flavonoids, tocopherols, and small heat shock proteins (Farrant and Moore 2011; Sano et al. 2016; Leprince et al. 2017).

The induction of proteins less prone to denaturation is possibly another way to alleviate the consequences of water loss, in addition to the mechanisms described above. This may generate the overall adjustment of the proteome to make it more resilient to consequences of macromolecular crowding induced by cellular dehydration. This suggestion seems feasible since IDPs/IDRs possess characteristics that can be useful in such cellular context. Analogously, eukaryotic cells often contain different kinds of cellular bodies (such as P-bodies and stress granules) conformed by an overcrowded milieu of macromolecules, including proteins. The concentrations of the proteins that constitute them are considerably higher than those of the crowded cytoplasm and almost always contain IDPs (Uversky 2017).

It has been demonstrated that the anti-aggregation activity of some IDPs is consequence of physical interference, whereby IDPs decrease the encounter frequency of aggregating proteins (Goyal et al. 2005; Chakrabortee et al. 2012; Toxopeus et al. 2014; Cuevas-Velázquez et al. 2016; Liu et al. 2016). For example, some members of the Late Embryogenesis Abundant (LEA) proteins have been proposed to prevent the coalescence of unfolded proteins by filling the space between the encounter molecules, in a similar way to the steric stabilization of colloidal dispersion promoted by polymers (Tunnacliffe and Wise 2007; Hughes and Graether 2011). It is recognized that IDPs are more functionally versatile than globular proteins; they tend to be multi-functional and can accommodate different structurally diverse interactors (Tomba and Kovacs 2010; Tunnacliffe et al. 2010; Kragelund et al. 2012; Bugge et al. 2018). An example of a multi-functional (moonlighting) IDP is anhydrin, a protein induced by dehydration in an anhydrobiotic nematode, *Aphelenchus avenae*. This protein can act as chaperone as well as endonuclease (Chakrabortee et al. 2010). On the other hand, the radical-induced cell death 1, a key plant-specific signaling hub, interacts with several transcription factors associated with development and stress responses via interaction with the IDRs of the transcription factors (Olsen et al. 2005).

This versatility could be very important under a stress context, because it could represent an energetic saving at a moment when energy expenditure in the cell must be maintained as low as possible. This fact is clearly exemplified by some LEA proteins which reflect the functional adaptability given by intrinsic disorder. For example, a dehydrin from citrus plays several roles in response to abiotic stress, serving as reactive oxygen species scavenger, but can also act as metal ion chelator (Hara et al. 2003; Hara et al. 2004; Hara et al. 2005). The wide substrate specificity activities observed for some dehydrins have been attributed to its disordered nature. This feature distinguishes them from typical molecular chaperones, which generally have specific interactions with a small number of client proteins (Kovacs et al. 2008). Thus, its structural resilience to coalesce could allow some IDPs/IDRs to interact as hubs with many partners during conditions of cellular dehydration. Consistently with this is the fact that IDRs constitute over 50% of the chains of RNA chaperones and over 30% of the sequences of protein chaperones (Tompa and Csermely 2004). IDRs in chaperones probably act not only as regulatory/structural components, but might also help them to perform their activity, without compromising their own structure by avoiding undesirable hydrophobic interactions, since attaching disordered regions to ordered proteins or aggregation-prone sequences helps to protect them from aggregation and thus making them more soluble (Minde et al. 2013; Liu and Huang 2014). Some IDPs/IDRs can undergo disorder-to-order transitions when their environment changes, such as during desiccation and osmotic stress (Goyal et al. 2003; Shih et al. 2010; Mansouri et al. 2016) and perform activities other than when they are in their disordered state (Popova et al. 2011).

There is evidence to support the suggestion that water-limiting conditions favor the accumulation of protein disorder. For example, several desiccation-tolerant microorganisms have proteomes richer in IDRs than their mesophilic counterparts (Kriško et al. 2010). In the classic example of orthodox seeds, it is shown that desiccation conditions clearly favor the accumulation of high levels of LEA proteins and others hydrophilic heat stable proteins (Amara et al. 2012). These proteins accumulate sharply during the last stage of seed formation, when a natural seed desiccation takes place, and several of them are also induced during periods of water deficit in vegetative tissues. LEA proteins represent the most numerous examples of plant IDPs related to conditions inducing cellular dehydration such as drought, desiccation, cold, salt, or freezing stress (Roberts et al. 1993; Garay-Arroyo et al. 2000; Tunnacliffe et al. 2010; Bremer et al. 2017). Several mechanisms of actions have been proposed for LEA proteins, for example, their high disorder content allows some of them to sequester water and sugars into a tight hydrogen-bonded network to form stable hydrated gels alongside sugars (Sun et al. 2013). Other functions of LEA proteins include membrane

stabilization, enzyme protection, metal binding, and antioxidant activities (Boucher et al. 2010; Liu et al. 2011; Hundertmark et al. 2011; Li et al. 2017; Bremer et al. 2017).

Although not in plants, IDPs have recently been linked to the survival ability of tardigrades in extreme desiccation (Boothby et al. 2017). Tardigrades conform a group of cryptobiotic micrometazoan species many of which are capable to survive under extreme conditions by entering in an ametabolic state, and they can restore its metabolism upon rehydration (Møbjerg et al. 2011; Yamaguchi et al. 2012). Under anhydrobiotic conditions, tardigrades secrete abundant heat-soluble hydrophilic proteins, which seem to protect extracellular constituent and/or secretory organelles (Fukuda et al. 2017). Differential gene-expression analysis in several species of tardigrades identified upregulation of several transcripts encoding IDPs, suggesting that the IDPs-mediated protection might be evolutionarily conserved (Boothby et al. 2017; Chavali et al. 2017). These proteins vitrify when dried and form a glass-like matrix in a process that is thought to trap proteins within the pores of the amorphous matrix, protecting them from denaturation. Upon rehydration, this reversible glassy matrix is dissolved (Boothby et al. 2017). This mode of action resembles the model proposed for LEA proteins where these proteins aid in the formation and stability of an intracellular glassy state that is indispensable for the survival during the seed desiccation (Tunnacliffe et al. 2010). Furthermore, some studies showed a correlation between the induction of IDPs/IDRs and the desiccation during seed germination. These observations were made during germination of *Zea mays* (Amara et al. 2012) and *Medicago truncatula* (Larre et al. 2006) seeds and through desiccation onset of *Glycine max* radicles (Liu et al. 2017). At least in the latter, the relative content of IDPs in the radicles subjected to desiccation was positively regulated and significantly surpassed the genome-based prediction.

Those evidences suggest a selective pressure to accumulate IDPs/IDRs in plant tissues when water supply is compromised. This floating hypothesis has previously been rejected based on predictions using genome-based data alone (Chakrabortee et al. 2012). Genomic-based data estimates are unable to encompass the dynamic plasticity of a cell regulation network depending on the cell type, environmental stimuli, or developmental stage. For example, under this approach, a dried maize caryopsis must be assumed to have the same disordome as every other vegetative structure of the maize plant. They share the same genetic information after all, but it cannot be assumed that the structure of the disordome is the same between leaves or roots compared with the latent caryopsis. This approach is therefore potentially biased, and its assumptions must be carefully validated before drawing any conclusion.

Nevertheless, there is still the need for comprehensive experimental transcriptome/proteome-based data analysis of the

disordome regulation in plant tissues subjected to conditions that promote dehydration such as drought, salt, freeze, heat, and osmotic stresses. An interesting test would be to analyze if proteins that conform the disordome are coordinately regulated and subjected to co-evolution. If this is correct and a disordome implied in the response to water stress in plants does exist, it is probable that such feature is the result of a selective pressure over the disordome as a whole, that acts collectively on each protein that conforms it, regardless of whether such proteins share phylogenetic relationships or not.

Future perspectives

In plants, IDPs/IDRs involved in drought stress responses could be required not only for enhancing molecular interconnection via multi-partner and fast interactions, but also for adequate function of some proteins under the molecular crowding conditions imposed by water stress. More information on the relationship between conditions that induce water scarcity in plants and disordome regulation is needed. More evidence is required to propose a generalized model that describes how this regulation can be accomplished by the plant. The demonstration of the existence of this possible link could provide a new perspective about plants' responses to abiotic stresses. Experiments oriented to characterize global disordome adjustment in stressed plants could also shed light on this subject. Computational analysis oriented to understand disordome regulation can be very helpful for this purpose. More emphasis must be put on the need for analysis based on experimental-acquired data in order to construct a more comprehensive model about the role that the disordome could play as a possible regulatory interconnected entity. Interesting questions could arise on this subject in the near future.

Acknowledgments We would like to thank Dra. Alejandra A. Covarrubias and Dr. César Cuevas and MC. Gabriela Flores Vargas for their suggestions.

Funding information This work was supported by the Consejo Nacional de Ciencia y Tecnología Mexico (grant numbers: 215098, 155356, and 221208).

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