Intrinsically disordered proteins: controlled chaos or random walk

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Abstract

Traditional conventions that a protein’s sequence dictates its definitive, tertiary structure, and that this fixed structure provides the protein with the ability to carry out its designated role(s) are still correct but not for all proteins. Research over the past decade discovered that several key proteins possess intrinsically disordered regions (IDRs) that are crucial to their ability to perform specific functions and are observed clustered together within important classes of proteins. In this review, we aim to demonstrate how free energy landscapes, molecular dynamics simulations, and homology modeling are helpful in understanding key conformational dynamics of intrinsically disordered proteins (IDPs). Additionally, we use a list of predicted IDPs found in Arabidopsis to identify chromatin organizers and transcriptional regulators as being highly enriched in IDPs. Furthermore, we focus our attention to specific proteins within these families such as HAC5, EFS, ANAC019, ANAC013, and ANAC046. Future studies are needed to experimentally identify additional IDPs and their binding mechanisms.

Introduction

Over the past two decades, the world of proteomics has undergone a significant paradigm shift. The classical approach to the study of proteins depended on the adherence to the protein structure-function model, where each protein was composed of an amino acid sequence that lead to a static structure and function. With the discovery of intrinsically disordered regions (IDRs), researchers have developed new approaches and methodologies to better understand the unstructured and dynamic world of intrinsically disordered proteins (IDPs).¹ Although all domains of life contain IDPs, eukaryotic proteins tend to show a significant level of enrichment. It has been demonstrated that approximately 33% of eukaryotic proteins contain at least one long stretch of residues (30 or more) that code for an intrinsically disordered region,² and greater than 30% of eukaryotic proteins have 50 or more consecutive disordered residues.³ Proteome-wide analyses of multiple plants have shown that roughly 30% of a plant’s proteome is comprised of proteins containing at least one region with 50 or more disordered residues. It has been suggested that the pronounced occurrence of IDPs in plant genomes might be advantageous for them to mount effective cellular responses under varied biotic and abiotic environmental conditions. This increased frequency of IDPs in plants could also lead to a high level of phenotypic plasticity.⁴ Intrinsically disordered proteins are found in nearly every class of proteins within the eukaryotic proteome, including transcription factors, signaling proteins, and proteins involved in chromatin remodeling.

The intrinsically disordered regions of unstructured proteins serve multiple purposes under different environmental conditions. Given that proteins generally form macromolecular complexes to execute diverse cellular functions, proteins with unstructured regions can have a wide range of binding partners and might participate in multiple biological processes.⁵ In addition, it was also demonstrated that the speed of a protein’s ability to bind to its partner(s) increased drastically in proteins with intrinsically disordered regions when compared to their structured, globular counterparts.⁶ Intrinsically disordered regions known as linker regions can hold two globular portions of a protein in close proximity with each other, while still allowing a large amount of flexibility in their spatial relationship with one another.⁷ This perhaps allows a protein with IDRs to bind with several binding partners simultaneously under changing cellular states.

Researchers have created several tools in order to better understand IDPs and their functions. Computational analyses of free energy landscapes (mapping all possible conformations of an entity) are vital to experimentally defining an IDR. Typically, proteins fold into their stable state at a distinct trough of the free energy landscape. However, IDPs show multiple shallow troughs due the increased number of conformational states. Free energy landscapes, along with molecular dynamics studies and homology modeling, allow predicting the conformational dynamics of IDPs. In this review, we aim to highlight computational tools that are helpful in understanding key conformational dynamics of IDPs. Distinctively, we will focus on intrinsically disordered proteins within the plant Arabidopsis thaliana (Arabidopsis). Our Gene Ontology (GO) enrichment analysis determines that proteins families belonging to chromatin remodeling and transcriptional regulation are statistically enriched in IDPs. We will discuss the potential roles of these classes of proteins in diverse biological processes with specific examples.

Free energy landscape of intrinsically disordered proteins

Due to thermal fluctuation, proteins can exhibit various conformations. The occurrence of each conformation is presented by the topography of the free energy landscape. Natively structured proteins usually fold into a native structure at the bottom of a free energy
Molecular dynamic simulations and homology modeling

Intrinsically disordered proteins exhibit high levels of flexibility and span multiple different conformations. Molecular dynamics (MD) simulations provide a tool to computationally explore the conformational space and examine the dynamics of a protein over time. Although computer simulations rely on a series of cumulative approximations that can be erroneous, it has become a necessary tool in the research involving IDPs.

Combined with experimental studies, typically NMR spectroscopy, the structural ensemble of the free energy landscape of IDPs can be constructed using computer simulations. Ensemble restrained MD simulations constitute a useful and important tool for modeling IDPs.17-19 Constructing an ensemble based on a pre-determined structural library represents another way of completing the task. ENSEMBLE,20 Select (SAS),21 ASTEROIDS,22 and BEGR,23 are pieces of software falling into this category.

IDPs are considered to have high specificity and low affinity when interacting with binding, partner molecules. Mutations may harm this interaction. MD simulations can be used to study an array of mutations to predict the consequences and provide mechanistic explanations without performing experiments. Using computational alanine scanning, Massova et al.24 suggested an approach to probe protein-protein interactions and evaluated binding free energies. In their case, they applied the method to p53 and MDM2 binding system where p53 is intrinsically disordered, and their results show excellent agreement with experimental data.24 Homology modeling is a useful tool to predict and study the structure of a protein from a homologue protein, where the structure of the protein in question has not been solved experimentally and the structure of its homologue protein is known. In the Arabidopsis genome, the COR15A gene is paired with COR15B.25 Proteins encoded by these two genes are homologues with 70% identity in their amino acid sequences. Overexpression of the COR15A gene in Arabidopsis produces excess mature COR15A protein in the chloroplast stroma,26 leading to enhanced freezing tolerance of chloroplasts of intact leaves and of isolated protoplasts frozen and thawed in vitro.26,27 In contrast, functional or structural information has been reported for COR15B protein. Both proteins show homology to the Pfam LEA_4 family of LEA proteins and both were predicted to be IDPs.28 Thalhammer et al.29 showed the structural modeling of these two proteins known LEA homologues and demonstrated the interactions between MGDC and the COR15 proteins that may help keep the cell integrity through freezing stress.

**Arabidopsis protein families enriched in intrinsically disordered proteins**

A recent genome-wide analysis was performed to predict IDRs in Arabidopsis and compare them with the human proteome.
Intriguingly, it was discovered that specific functional classes are enriched with IDRs in Arabidopsis. These functional groups include post-translational protein modification and response to red or far red light. While these broad functional classes provided insightful clues on the essential roles of these IDPs/IDRs in Arabidopsis, here we reevaluated these data to specifically predict protein families enriched with IDRs. To achieve this, we compiled a list of IDPs containing at least five long disordered regions (>30 residues). We subjected these IDPs to functional annotation tool, DAVID (the Database for Annotation, Visualization and Integrated Discovery) to predict the statistically enriched GO categories as well as enriched groups of different protein families (Supplementary Table S1: Functional Annotation of Predicted IDPs). DAVID employs a novel agglomeration algorithm to assemble a list of genes or associated biological terms into organized classes of related genes. Subsequently, we used the PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification tool to organize the lists by molecular function (Figure 2).

Chromatin remodeling

Even though cells within most eukaryotic organisms specialize in both structure and function, each contains the same genomic DNA of the organism. Therefore, the uniqueness of each cell is derived not by the DNA sequence but instead by the availability of portions of the DNA within that particular cell. The accessibility of genes is regulated not only through the prevalence of transcription factors and enhancers, but also through the structure of the DNA itself. The DNA of eukaryotes is associated with histone octamers that sequester approximately 147 base pairs of DNA in a nucleosome complex. Histone octamers are eight-protein complexes consisting of two copies of four subunits (H2A, H2B, H3, and H4). The DNA that is associated with histone proteins is not accessible to the cellular machinery responsible for transcription, chromatin assembly, DNA repair, and a variety of other processes. The tightness of this association is

Figure 2. Classification of enrichment groups by molecular function. The two enriched groups, Transcription Regulation (A) and Chromatin Organization (B), are organized by their molecular function using the PANTHER classification tool.

Figure 3. Chromatin organization through transferases. The transition between euchromatin and heterochromatin can be facilitated by various transferases. Acetylation of histones leads to the relaxation of chromatin structure. Histone methylation is more complex and depends on the location and extent of methylation.

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Heterochromatin occurs when the histone proteins disassociate with the DNA completely or spread apart, decreasing the amount of DNA directly associated with histones. The structure of chromatin can be dynamic, and it is regulated by a group of proteins and complexes known as chromatin remodelers. These chromatin remodelers can modify the histone octamers in a variety of ways including acetylation, ubiquination, phosphorylation, methylation, and sumoylation. The histone modifications either relax or tighten the structure of the localized DNA thereby permitting or restricting the transcription of nearby genes. Histone acetylation causes the relaxation of chromatin, heterochromatin, and euchromatin. Heterochromatin occurs when the histone proteins disassociate with the DNA completely or spread apart, decreasing the amount of DNA directly associated with histones.

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Transcription factors

Transcription factors are modular proteins that contain one or more DNA-binding domains, recognize and bind to specific DNA sequences, therefore regulating the rate of transcription of genetic information from DNA to RNA. Generally, a prototypical transcription factor contains a DNA-binding domain (DBS), signal-sensing domain (SSD), and a transactivation domain (TAD). And the two processes involved in transcriptional regulations: protein-protein interaction and protein-nucleotide interaction have been reported to be accompanied often by a local folding in a protein molecule, which may adjust the transcription factor flexibility correspondingly.}

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pathway, suggesting that it may participate in the transmission of cryptochrome (CRY1 and CRY2) signals. In darkness, the degradation of HY5 inhibits the activation of light-induced genes. It has been reported that HY5 is negatively regulated by COP1, a light-inactivatable repressor of photomorphogenetic development interacting directly and specifically with HY5.47 Interestingly, the plant hormone cytokinin also induces similar phenotypes as the cryptochrome flacin-type photoreceptors. hy5 mutants show a reduced induction of anthocyanin accumulation in blue light by cytokinins. It has been shown that cytokinins can increase the levels of HY5 protein accumulation, hinting that cytokinin could play a role in stabilizing HY5 protein, and that the regulation of HY5 stability could act at intersection of cytokinin signaling pathway and cryptochrome pathways.48 Abscisic acid (ABA), another phytohormone, regulates seed germination and seedling development as light. It was found that HY5 binds to the promoter of the transcription factor AB15, which is significantly enhanced by ABA, while overexpression of AB15 led to increased light response.49

NAC (NAM, ATAF, CUC) transcription factors share an N-terminal NAC domain and regulate stress perception and developmental programs. The crystal structure of the ANAC019 NAC domain consists of twisted -sheet packing against a -helix on both sides.50,51 The NAC transcription regulatory domains (TRDs) contain group-specific sequence motifs and have a high degree of intrinsic disorder.52 Both full-length and truncated ANAC019 are able to induce the expression of stress-responsive marker genes [COR47 (cold-responsive 47), RD29b (responsive-to-desiccation 29b) and ERD11 (early-responsive-todehydration11)]. Replacing the NAC domain of ANAC019 with the analogous regions from other NAC transcription factors still keeps the ability to regulate ABA signaling, while replacing the ANAC019 TRD with other TRDs loses the ABA signaling regulation ability.52 Further it has been shown that ANAC019 interacts with the RING-finger H2-type E3 ubiquitin-protein ligase RHA2a.53 ANAC019 may play a dual role in regulating ABA and jasmonate response with the other RHA2a-interacting protein ANAC055. These two signaling pathways are involved in the activation of defense responses to both biotic and abiotic stresses, and ANAC019 and ANAC055 could serve as players linking the crosstalk between these two signaling pathways (Figure 4).54 Additionally, two other NAC proteins, ANAC046 and ANAC013, have recently been experimentally identified as IDPs. It has been shown that both of these aforementioned NAC transcription factors are capable of interacting with the small hub protein known as Radical-induced Cell Death 1 (RCD1), at least partially due to their intrinsically disordered region. Both proteins are involved in plant senescence. Interestingly, the interaction between the two TFs and RCD1 does not appear to be dependent on a disorder-to-order transition.54 Future research could focus on determining if ANAC019 interacts with its binding partners in a similar way.

Conclusions

The discovery of intrinsically disordered proteins created a novel area of proteomics. Specifically, the increased knowledge of protein folding dynamics may lead to a better understanding of plants' phenotypic plasticity. Because plants are fixed in soil and unable to move, they create complex mechanisms for coping with biotic and abiotic environmental stresses. The concept of flexible proteins, which are able to change their interaction profile based on cellular conditions, leads to a new way of thinking about plant plasticity. We have highlighted tools that are useful for characterizing IDPs' conformational dynamics. Additionally, we have demonstrated that Arabidopsis contains two highly enriched groups of IDPs; both of with are key components in the transcriptional regulation landscape. Further studies need to focus on experimentally verifying proteins as IDPs, and describing the advantages and disadvantages of intrinsically disordered regions over their more structured counterparts. Furthermore, IDPs have been recent targets for therapeutic strategies for mammalian diseases such as Parkinson's disease.55 Similarly, IDPs found in plants could be targets for decreasing crops' susceptibility to disease or increasing overall yield. Additional research could focus on identifying key IDP targets for both therapeutics and plant resistance.

References

1. Tompa P. Intrinsically unstructured proteins. Trends Biochem Sci 2002;27:527-3.
2. Ward JJ, Sodhi JS, McGuffin LJ, et al. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J Mol Biol 2004;337:635-45.
3. Dunker AK, Lawson JD, Brown CJ, et al. Intrinsically disordered protein. J Mol Graph Model 2001;19:26-59.
4. Marin M, Ott T. Intrinsic disorder in plant proteins and phytopathogenic bacterial effectors. Chem Rev 2014;114:6912-32.
5. Wright PE, Dyson HJ. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. J Mol Biol 1999;293:321-31.
6. Huang Y, Liu Z. Kinetic advantage of intrinsically disordered proteins in coupled folding-binding process: a critical assessment of the fly-casting mechanism. J Mol Biol 2009;393:1143-59.
7. Tantos A, Han KH, Tompa P. Intrinsic disorder in cell signaling and gene transcription. Mol Cell Endocrinol 2012;348:457-65.
8. Dunker AK, Oldfield CJ, Meng J, et al. The unfoldomics decade: an update on intrinsically disordered proteins. BMC Genomics 2008;9:S1.
9. Higo J, Umezawa K. Free-energy landscape of intrinsically disordered proteins investigated by all-atom multicanonical molecular dynamics. Adv Exp Med Biol 2014;805:331-51.
10. Jensen MR, Zweckstetter M, Huang JR, Blackledge M. Exploring free-energy landscapes of intrinsically disordered proteins at atomic resolution using NMR spectroscopy. Chem Rev 2014;114:6632-60.
11. Uversky VN. Natively unfolded proteins: a point where biology waits for physics. Protein Sci 2002;11:739-56.
12. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. Nat Rev Mol Cell Biol 2005;6:197-208.
13. Uversky VN, Dunker AK. Understanding protein non-folding. Biochim Biophys Acta 2010;1804:1231-64.
14. Metallo SJ. Intrinsically disordered proteins are potential drug targets. Curr Opin Chem Biol 2010;14:481-8.
15. Uversky VN. Targeting intrinsically disordered proteins in neurodegenerative and protein dysfunction diseases: another illustration of the D(2) concept. Expert Rev Proteomics 2010;7:543-64.
16. Wang J, Cao Z, Zhao L, Li S. Novel strategies for drug discovery based on intrinsically disordered proteins (IDPs). Int J Mol Sci 2011;12:3205-19.
17. Ganguly D, Chen J. Structural interpretation of paramagnetic relaxation enhancement-derivered distances for disordered protein states. J Mol Biol 2009;390:467-77.
18. Allison JR, Varnai P, Dobson CM, Vendrusculo M. Determination of the free energy landscape of alpha-synuclein using spin label nuclear magnetic resonance measurements. J Am Chem Soc 2009;131:1831-46.
19. Lindorff-Larsen K, Best RB, Depristo MA, et al. Simultaneous determination of protein structure and dynamics. Nature 2005;433:128-32.
20. Krzemiński M, Marsh JA, Neale C, et al. Characterization of disordered proteins with ENSEMBLE. Bioinformatics 2013;29:398-9.
21. Chen Y, Campbell SL, Dokholyan NV. Deciphering protein dynamics from NMR
data using explicit structure sampling and selection. Biophys J 2007;93:2300-6.

22 Nodet, G., Salmon, L., Ozenne, V., et al. Quantitative description of backbone conformational sampling of unfolded proteins at amino acid resolution from NMR residual dipolar couplings. J Am Chem Soc 2009;131:17908-18.

23 Daughdrill, GW, Kashaetrov, S, Staniek, A, et al. Understanding the structural ensembles of a highly extended disordered protein. Mol Biosyst 2012;8:308-19.

24 Massova, I, Kollman, P. Computational ala scanning to probe protein-protein interactions: a binding free energies. J Am Chem Soc 2013;135:2304-15.

25 Wilhelm, KS, Thomas, M. Arabidopsis thaliana cor15b, an apparent homologue of cor15a, is strongly responsive to cold and ABA, but not drought. Plant Mol Biol 1993;23:1073-7.

26 Artus, NN, Uemura, M, Steponkus, PL, et al. Constitutive expression of the cold-regulated Arabidopsis thaliana COR15A gene affects both chloroplast and proplastid freezing tolerance. Proc Natl Acad Sci USA 1996;93:10404-9.

27 Steponkus, PL, Uemura, M, Joseph, RA, et al. Mode of action of the COR15A gene on the freezing tolerance of Arabidopsis thaliana. Proc Natl Acad Sci USA 1998;95:14570-5.

28 Hundtemark, M, Hincha, DK. LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana. BMC Genomics 2008;9:118.

29 Thalhammer, A, Hundtemark, M, Popova, AV, et al. Interaction of two intrinsically disordered plant stress proteins (COR15A and COR15B) with lipid membranes in the dry state. Biochim Biophys Acta 2010;1798:1812-20.

30 Pietrosemoli, N, Garcia-Martin, JA, Solano, R, Pazos, F. Genome-wide analysis of protein disorder in Arabidopsis thaliana: implications for plant environmental adaptation. PLoS One 2013;8:e55524.

31 Huang, da W, Sherman, BT, Lempicki, RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1-13.

32 Mi, H, Muruganujan, A, Thomas, PD. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res 2013;41:D377-86.

33 Mi, H, Muruganujan, A, Casagrande, JT, Thomas, PD. Large-scale gene function analysis with the PANTHER classification system. Nat Proteo 2013;8:1551-66.

34 Zhou, Y, Kim, J, Yuan, X, Braun, T. Epigenetic modifications of stem cells: a paradigm for the control of cardiac progenitor cells. Circ Res 2011;109:1067-81.

35 Marino-Ramirez, L, Kann, MG, Shoemaker, BA, Landsman, D. Histone structure and nucleosome stability. Expert Rev Proteomics 2005;2:719-29.

36 Gorisch, SM, Wachsmuth, M, Toth, KF, et al. Histone acetylation increases chromatin accessibility. J Cell Sci 2005;118:5825-34.

37 Pandey, R, Muller, A, Napoli, CA, et al. Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversification of chromatin modification among multicellular eukaryotes. Nucleic Acids Res 2002;30:5036-55.

38 Bordoli, L, Nitsch, M, Luthi, U, et al. Plant orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related proteins. Nucleic Acids Res 2001;29:589-97.

39 Li, C, Xu, J, Li, J, et al. Involvement of Arabidopsis histone acetyltransferase HAC family genes in the ethylene signaling pathway. Plant Cell Physiol 2014;55:426-35.

40 Li, C, Xu, J, Li, J, et al. Involvement of Arabidopsis HAC family genes in pleiotropic developmental processes. Plant Signal Behav 2014;9:e28173.

41 Han, SK, Song, JD, Noh, YS, Noh, B. Role of plant CBP/p300-like genes in the regulation of flowering time. Plant J 2007;49:103-14.

42 Berr, A, Xu, L, Gao, J, et al. Set domain group25 encodes a histone methyltransferase and is involved in flowering locus C activation and repression of flowering. Plant Physiol 2009;151:1476-85.

43 Santos-Rosa, H, Schneider, R, Bannister, AJ, et al. Active genes are tri-methylated at K4 of histone H3. Nature 2002;419:407-11.

44 Shafiq, S, Berr, A, Shen, WH. Combinatorial functions of diverse histone methylations in Arabidopsis thaliana flowering time regulation. New Phytol 2014;201:312-22.

45 Spolar, RS, Record, MT, Jr. Coupling of local folding to site-specific binding of proteins to DNA. Science 1994;263:777-84.

46 Liu, J, Perumal, NB, Oldfield, CJ, et al. Intrinsic disorder in transcription factors. Biochemistry 2006;45:6873-88.

47 Ang, LH, Chattopadhyay, S, Wei, N, et al. Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. Mol Cell 1998;1:213-22.

48 Vandenbussche, F, Habricot, Y, Condiff, AS, et al. HY5 is a point of convergence between cryptochrome and cytokinin signaling pathways in Arabidopsis thaliana. Plant J 2007;49:428-41.

49 Chen, H, Zhang, J, Neff, MM, et al. Integration of light and abscisic acid signaling during seed germination and early seedling development. Proc Natl Acad Sci USA 2008;105: 4495-500.

50 Ernst, HA, Olsen, AN, Larsen, S, Lo Leggio, L. Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. EMBO Rep 2004;5:297-303.

51 Greve, K, La Cour, T, Jensen, MK, et al. Interactions between plant RING-H2 and plant-specific NAC (NAM/ATAF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization. Biochem J 2003;371:97-108.

52 Jensen, MK, Kjaersgaard, T, Nielsen, MM, et al. The Arabidopsis thaliana NAC transcription factor family: structure-function relationships and determinants of ANAC019 stress signalling. Biochem J 2010;426:183-96.

53 Jiang, H, Li, H, Bu, Q, Li, C. The RHA2a-interacting proteins ANAC019 and ANAC055 may play a dual role in regulating ABA response and jasmonate response. Plant Signal Behav 2009;4:464-6.

54 O’Shea, C, Kryger, M, Stender, EG, et al. Protein intrinsic disorder in Arabidopsis NAC transcription factors: transcriptional activation by ANAC013 and ANAC046 and their interactions with RCD1. Biochem J 2015;465:281-94.

55 Toth, G, Gardai, SJ, Zago, W, et al. Targeting the intrinsically disordered structural ensemble of alpha-synuclein by small molecules as a potential therapeutic strategy for Parkinson’s disease. PLoS One 2014;9; e87133.