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The disordered C-terminus of the RNA Polymerase II phosphatase FCP1 is partially helical in the unbound state

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ABSTRACT

Intrinsically disordered proteins (IDPs) lack unique 3D structures under native conditions and yet retain critical functions. Recycling of RNA Polymerase II after transcription is promoted by an interaction between the winged helix domain of RAP74, a component of the general transcription factor IIF (TFIIF), and the C-terminus of the TFIIF-associating CTD phosphatase (FCP1). Sixteen residues from the C-terminus of FCP1 form an α-helix in the complex, but the protein is otherwise agreed in the literature to be intrinsically disordered. Here we show through CD and recently developed carbon-detected NMR that, although FCP1 is intrinsically disordered, the above 16 residues composing the RAP74 binding surface form nascent α-helical structure in the unbound state. We further show retention of general FCP1 disorder and the nascent helical content in HeLa extract, establishing cellular relevance. The conformational bias observed leads to a mechanistic proposal for FCP1's transition from a disordered ensemble to an ordered conformation upon binding.

1. Introduction

A protein's function is generally assumed to be a property of its stable and unique 3D structure. In contrast, intrinsically disordered proteins (IDPs) are over-represented in transcription, translation, and cellular signaling; having emerged as critical components of these cellular systems [1,2]. The molecular details of disorder-to-order transitions in IDPs have been the subject of intense recent study [3–5], because IDPs tend to fold upon interaction with a binding partner (typically another protein or a nucleic acid).

FCP1 (TFIIF associating component of the RNA Polymerase II C-terminal domain phosphatase) features an approximately 80 residue IDP segment at its C-terminus that is vital for its recruitment to and regulation of RNA Polymerase II (RNAPII) at the termination of transcription. The C-terminal repeat domain (CTD) of RNAPII undergoes a cycle of phosphorylation and dephosphorylation as the polymerase progresses through the messenger RNA biogenesis cycle [6]. Interaction between the C-terminal acidic domain of FCP1 (residues 879–961; further referred to as ctFCP1) with the C-terminal winged helix domain of the RAP74 chain of TFIIF promotes FCP1 activity [7]. The structure of ctFCP1 bound to RAP74 has been determined by crystallography [8] and NMR spectroscopy [9], revealing an α-helical conformation for residues 944–960. The remainder of ctFCP1 is presumed to be disordered in the RAP74 bound complex and, similarly, it has been suggested ctFCP1 is entirely disordered when free in solution [9].

Unlike their globular counterparts, IDPs offer only limited NMR structural constraints. As a result, the structural information encoded in the chemical shift itself has emerged as a key indicator of IDP ensemble properties [10,11]. Additional structural inferences can be drawn from chemical shift perturbations induced by osmolytes, chaotropes, and crowding agents [12–14]. While NMR based IDP structure studies have begun to emerge [15,16], application of the technique is still limited due to its challenging nature [17,18]. Recent advances from our lab and others have resulted in the re-emergence of ^{13}C-direct detection spectroscopy as a tool for studying proteins in solution [19,20], with substantial advantages in resolution and sensitivity realized for IDPs. Here we report a chemical shift based structural characterization of ctFCP1 in three states: free in solution, bound to RAP74, and perturbed by co-solutes. These novel studies show that the RAP74 binding region of ctFCP1 is partially helical free in solution, while the remainder of the domain is strongly disordered and remains so when bound to RAP74 or when in the presence of HeLa cell extract. Implications for folding-upon-binding mechanisms are discussed.

Abbreviations: CSI, chemical shift index; FCP1, transcription factor IIF associating CTD phosphatase 1; IDP, intrinsically disordered protein; TFE, 2,2,2-trifluoroethanol.

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2. Materials and methods

2.1. Protein preparation

The human ctFCP1 samples (corresponding to residues 879–961 of the full protein) for CD and NMR spectroscopy were prepared and purified as previously reported [21], except that they were lyophilized at the completion of the purification process. The RAP74 winged helix domain was purified through the same protocol as ctFCP1. The CD samples were brought to a final concentration of 20 μM FCP1 in 20 mM cacodylate, pH 7.0, with 2,2,2-trifluoroethanol (TFE) titrated as described. Additional CD experiments were performed using a synthetic peptide comprised of FCP1 residues 944–961 purchased from the Tufts University Core Facility and similarly dissolved from lyophilized powder. The ^{15}N,^{13}C labeled NMR samples were 1 mM ctFCP1 in 20 mM sodium phosphate, pH 7.0, 100 mM NaCl, 0.02% (w/v) NaN_3, 10% (v/v) D_2O. Separate NMR samples were generated in which TFE, urea, dextran, or HeLa extract were added to the concentrations indicated in each experiment.

2.2. Circular dichroism

CD was used to observe the effect of TFE on the FCP1 peptide and ctFCP1. All CD data were collected on a Jasco J-810 spectropolarimeter. Measurements were taken from 205 to 250 nm every 1 nm with a bandwidth of 2 nm. TFE concentrations ranged from 0% to 40% (v/v) in increments of 10%.

2.3. NMR spectroscopy

All experiments were recorded on an 11.7 T Bruker AVANCE-3 spectrometer operating at 500.13 MHz ^1H frequency equipped with a TCI cryoprobe, allowing high sensitivity acquisition of ^{13}C-direct detected spectra. All spectra were recorded at 298 K. ^{15}N,^{13}C-CON-IPAP spectra were collected using the standard version of the pulse sequence found in the Topspin 2.1 library. Following virtual decoupling of the specta in Topspin, all NMR spectra were processed in NMRPipe [22]. Peak picking and resonance assignment was done in Sparky [23].

Analysis of secondary structure through chemical shifts of backbone ^{13}C atoms was performed using the standard chemical shift indexing (CSI) method and in-house software [24]. The change of 2D peak position in ^{15}N,^{13}C-CON spectra was used as an indication of chemical environment changes in response to RAP74 or co-solute addition and is quantified as:

$$\Delta\delta = \sqrt{(N\Delta_{\mathrm{ppm}}\alpha)^2 + (CO\Delta_{\mathrm{ppm}})^2}$$

where Δ_{ppm} is the change in chemical shift between the two states and $\alpha = 0.3$ normalizes the ^{15}N and ^{13}C chemical shift ranges. TFE was titrated in increments of 5% (v/v) from 0% to 40%. Urea was titrated in increments of 0.5 M from 0.0 to 3.0 M. Measurements of crowding effects were made through the mixture of 30% (w/v) dextran or 70 mg/mL HeLa cell extract with sufficient aqueous buffer to generate a standard 500 μL sample. The RAP74 bound sample was generated by mixing ctFCP1 and RAP74 winged helix domain in a 1:1.2 M ratio in standard NMR buffer.

3. Results and discussion

3.1. Structural response of ctFCP1 to RAP74 binding

Disordered protein segments often mediate protein–protein interactions, as is evidenced by the known interaction between ctFCP1 and the winged helix domain of RAP74. Recent advances in bioinformatics methods have increased the number of predicted interactions, while experimental validation has often lagged behind. Analysis of ctFCP1 using the ANCHOR [25] program predicts two regions with a high likelihood of forming protein–protein contacts (Fig. 1A, indicated by bars over the graph), one of which is the known RAP74 binding site. In prior studies of ctFCP1 bound to RAP74, nearly all of the resonances from the N-terminus of the construct to around residue 920 were unassigned due to severe overlap or absence from 2D ^{15}N,^1H-HSQC spectra [9]. It is therefore possible that the second predicted interaction site may also (at least transiently) interact with RAP74.

Incomplete chemical shift assignments are common in ^1H-detected NMR of IDPs. To circumvent this problem, we have recently begun comprehensively employing ^{13}C-direct detection NMR methods in our studies of IDPs. These methods are especially effective when the carbonyl carbon is chosen as the detection nucleus because its high chemical shift anisotropy renders its isotropic chemical shift sensitive to even small variations in local structure. Using ^{13}C-detected spectroscopy, we have generated comprehensive chemical shift assignments for ctFCP1 in the free and RAP74 bound states [21]. Comparison of 2D peak positions in the ^{15}N,^{13}C-CON spectra of apo-ctFCP1 and the ctFCP1-RAP74 complex shows clearly that the only detectable contacts with RAP74 are

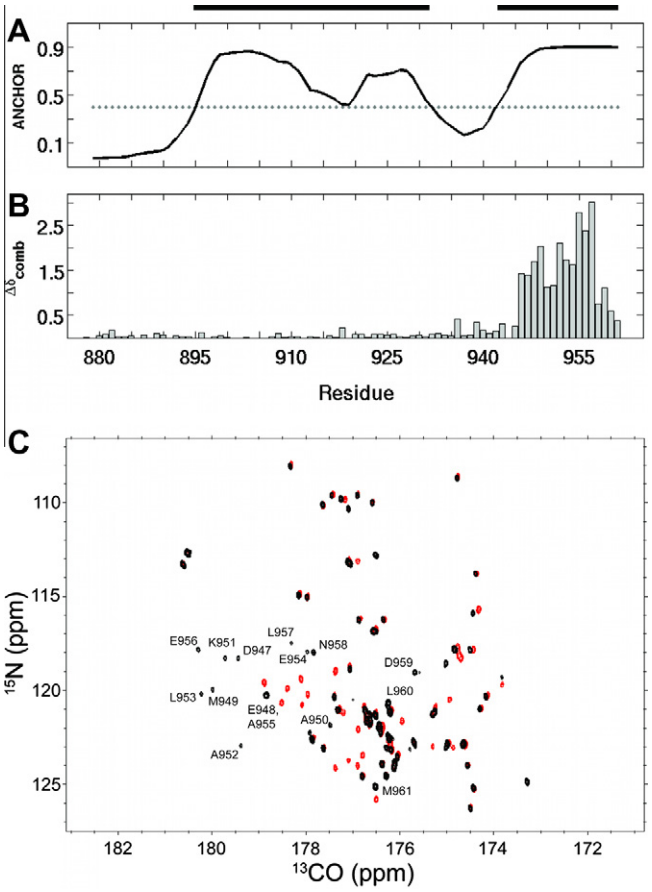

Fig. 1. Structural response of ctFCP1 to RAP74 binding. (A) ANCHOR[28] analysis indicates that there are two potential regions of protein–protein interaction in ctFCP1 (indicated with bars above the figure). (B) The most C-terminal predicted binding site corresponds to the known RAP74 interaction region, which is the only portion of ctFCP1 to display significant backbone chemical shift changes upon RAP74 binding. (C) The ^{15}N,^{13}C-CON spectra of apo-ctFCP1 (red) and the ctFCP1-RAP74 complex (black) are overlaid and zoomed to highlight the resonances from the RAP74 interaction region (D947-M961; labeled in the bound state). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

those formed by the previously identified residues 944–961 (Fig. 1B and C). The extraordinary spectral quality of the ^{15}N,^{13}C-CON spectrum and well dispersed ctFCP1 resonances are clearly seen in Fig. 1C, which is zoomed to highlight the resonances that shift in response to RAP74 binding. We are currently screening for other proteins associated with the RNAPII termination complex that may form contacts mediated by the second ANCHOR predicted interaction region.

It is well documented that disordered protein segments often transiently sample secondary structures that they will adopt in complex while free in solution [5]. Given the known helical structure of ctFCP1 in complex with RAP74, we explored the possibility of its pre-formation in the apo-state through application of the ^{13}C chemical shift index (CSI; Fig. 2) [24]. Consensus analysis of chemical shifts indicates strong enough secondary shifting to assign a helical conformation to residues 945–949, which are found in the N-terminal portion of the RAP74 binding helix. The analysis indicates that the remainder of ctFCP1 is in a disordered state, although there is significant tendency towards helical chemical shifts in many other residues composing the RAP74 binding interface as well. The detection of a short stretch with helical tendencies in the unbound state is contrary to previous observations [9] and attributed here to the improved completeness of chemical shift assignment of the unbound state achieved with ^{13}C-detected methods. We are unaware of any previous analysis of ctFCP1 comparable to ours, leading here to the first observation of nascent helical structure in the RAP74 interaction region while unbound.

3.2. Circular dichroism

Having identified by NMR a tendency in apo-ctFCP1 to nucleate α-helical structure, we next chose to validate the finding with a second technique. Unlike NMR, CD spectra are averaged over the entire length of the polypeptide and the ~60 residues displaying random structure that precede the helical region may make it difficult to detect transient or non-cooperative helix formation in the binding region. We therefore chose to measure circular dichroism using both the ctFCP1 construct from the NMR samples and a shorter synthetic peptide (comprising residues 944–961) in which the fraction of helical residues should be enriched. The spectra for the peptide and for ctFCP1 are shown black in Fig. 3A and B, respectively. While both are characteristic of CD spectra for IDPs, they also feature a subtle minimum near 222 nm – the hallmark of an α-helix. Even in the shortened peptide, however, the helical character of the spectrum was very minimal. These data are consistent with either a short region with helical tendency, as was observed by NMR, or with only transient and non-cooperative helix formation.

In nature, small compatible co-solutes known as osmolytes are often employed to stabilize the folded state of proteins in the presence of dehydration stress [12]. As a result, osmolytes are often used as co-solutes to provide additional information in protein folding studies and, more recently, for inducing folding in IDPs [14]. After an initial screening process, we found 2,2,2-trifluoroethanol (TFE) to increase the clarity and depth of the CD minimum at 222 nm in both the peptide and the ctFCP1 spectra (Fig. 3, colored curves). The CD experiments with ctFCP1 show a similar increase in α-helicity to the shorter peptide, but the transition does not feature as clear of a minimum at 222 nm; most likely because convolution with signal from the larger disordered region is masking the helical signal from the binding region.

3.3. NMR titrations with TFE and urea

The CD data provided evidence that TFE can be used to fold ctFCP1 and, possibly, to see an intermediate on the RAP74 binding pathway. CD provides a representation of helical content averaged over the entire backbone, so it is not clear how best to interpret the signal. It was chemical shift index analysis that initially led to the observation of residue specific native helical tendency in apo-ctFCP1 (Fig. 2). Therefore, we returned to monitoring NMR chemi-

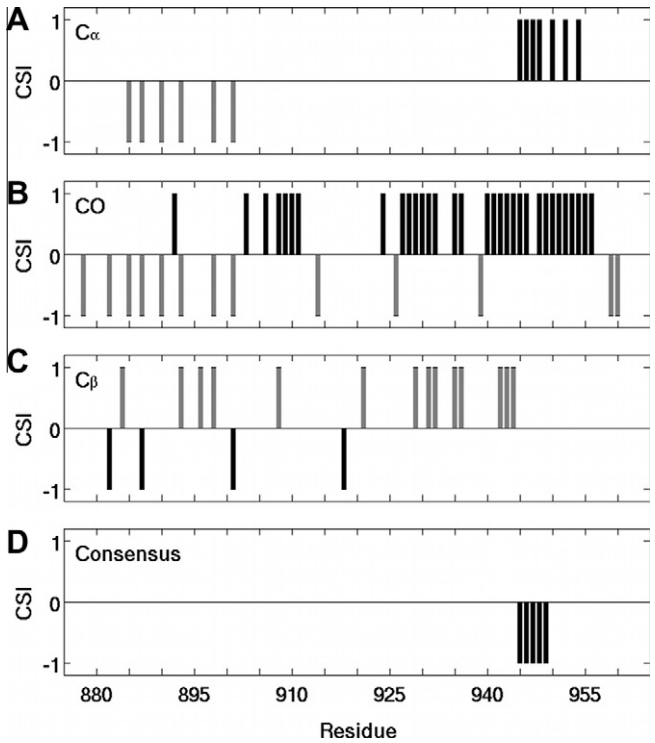

Fig. 2. Chemical shift index (CSI) analysis of ctFCP1. Analysis of $^{13}C\alpha$, ^{13}CO, and $^{13}C\beta$ chemical shifts provides a consensus indication of secondary structure in ctFCP1 that suggests helix nucleation in the RAP74 binding region may occur in the unbound state. Chemical shifts consistent with α-helix are shown with black bars, whereas those consistent with extended or β-strand structure are shown in gray.

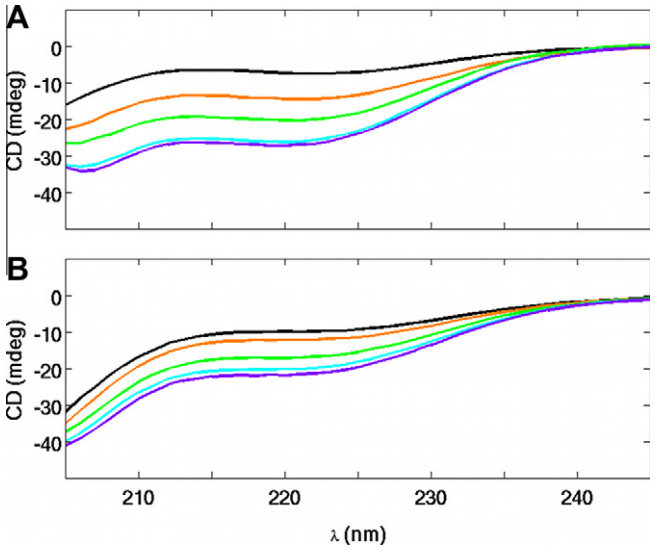

Fig. 3. TFE-dependent conformational transitions in ctFCP1 monitored by circular dichroism. CD spectra of (A) FCP1 (944–961) peptide and (B) ctFCP1 (879–961) in 0% (black), 10% (orange), 20% (green), 30% (blue) and 40% (purple) TFE (v/v). Increasingly negative CD signal with a minimum near 222 nm is indicative of increased α-helical structure as the TFE volume percent increases.

cal shift changes for ctFCP1 as TFE was titrated from 0% to 40% (v/v). A full CSI analysis was not possible at TFE concentrations greater than 10% because the sample lifetime was too short for 3D spectra to be acquired. Fig. 4A displays in color the difference in ^{13}CO chemical shift from the pure aqueous state for each residue as TFE is titrated in. The ^{13}CO chemical shift, taken directly from the 2D spectra, was chosen as a reporter due to its extreme sensitivity to small changes in local environment – not just to the formation of stable secondary structure – providing comprehensive detail regarding TFE induced effects.

The ^{13}CO shift data displays a clear trend towards increasingly positive deviation for residues in the RAP74 binding region, indicating enhanced α-helix stability. A systematic decrease indicating the loss of helicity, or a transition towards a more extended structure, is not observed in any region of the protein. Given that chemical shift indexing had already provided evidence of helical tendency in the RAP74 interaction region, we chose to also titrate urea from 0 to 3 M and monitor the same ^{13}CO chemical shift deviation from the aqueous reference for each amino acid (Fig. 4A, grayscale). As with TFE, urea was found to have large and systematic effects only in the binding region. These chemical shift changes trended in the opposite direction from those induced by the presence of TFE, confirming a loss of helical structure. Chemical shift changes throughout the rest of the protein were small and random, indicating little to no overall effect from urea addition. In connection with the CSI analysis, these two titrations collectively demonstrate a native tendency towards α-helical secondary structure in the RAP74 interaction region of apo-ctFCP1 in aqueous solution.

3.4. ctFCP1 remains disordered in environments mimicking cellular crowding

Given the implications for RAP74 binding of finding nascent helical structure in apo-ctFCP1, we wanted to confirm that the conformation studied in homogeneous dilute solution is representative of what would be present in the cell. Polymeric crowding agents have been used to good effect as mimics of cellular crowding to study protein folding, binding affinities, and association kinetics [13]. Dextran is widely regarded as an inert polymer with respect to protein interactions and at 30% (w/v) should mimic well the extent of crowding and confinement expected under cellular conditions with minimal risk of contamination from specific protein–polymer interactions [26]. The 2D peak position of each ^{15}N,^{13}C-CON resonance showed virtually no change in 30% dextran, relative to the dilute aqueous state (Fig. 4B). The small variations observed are likely attributable to subtle changes in solvent dielectric or other physical parameters. What is clear from the very small magnitude of the change is that ctFCP1 neither folds into a more orderly structure imparting large chemical shift changes, nor experiences a change in the position of the helix-coil equilibrium in the RAP74 binding region upon crowding.

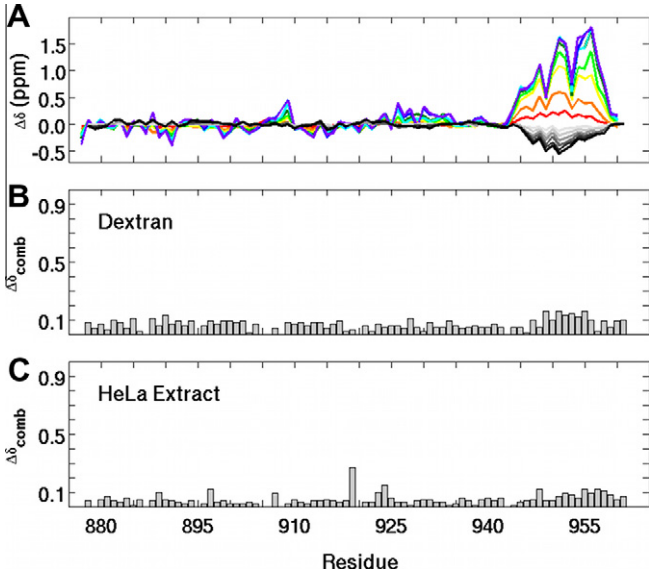

Fig. 4. Structural response of ctFCP1 to co-solute addition. (A) Per-residue ctFCP1 ^{13}CO chemical shift changes during TFE titration (progressing 5% red, 10% orange,15% yellow, 20% light green, 25% dark green, 30% light blue, 35% dark blue, to 40% purple) indicate increasing helicity. The response to urea titration (increasing from 0.5 M in light gray to 3.0 M in black with 0.5 M increments) indicates decreasing helicity. The backbone chemical shifts of ctFCP1 are unresponsive to the addition of (B) 30% (w/v) dextran or (C) 70 mg/mL HeLa cellular extract, indicating that ctFCP1 remains disordered even in highly crowded and cell mimicking conditions.

As a further demonstration that the conformations monitored in our NMR samples are physiologically relevant, we next collected a ^{15}N,^{13}C-CON spectrum in the presence of 70 mg/mL HeLa cell extract. Again, the change in 2D peak position was both extremely small and relatively uniform (Fig. 4C). The only site to show a chemical shift difference of even just 0.2 ppm was His-918, which is likely reporting on small changes in pH rather than a structural rearrangement of the ctFCP1 backbone. Protease degradation of the sample on the hours timescale prevented more detailed characterization. While it is surely the case that specific interactions with the RNAPII holoenzyme, in addition to interaction with the winged helix of RAP74, induce changes in the structure and dynamics of ctFCP1, we conclude from these studies that our NMR structural analysis presents a realistic picture of the ctFCP1 conformation that is encountered by RAP74 and the RNAPII machinery prior to binding.

3.5. Implications for the RAP74 binding mechanism

The long held paradigm in molecular biophysics that a protein's function is encoded uniquely in its 3D structure has been challenged by IDPs. Demonstration of IDP functional prevalence in signaling and, particularly, in regulating transcription has demanded the model's modification. It is now generally accepted that disordered 3D structure is as well suited to function as orderly, cooperatively folded, globular structure. Still, most IDPs are found to adopt one or more temporally stable and highly populated structures in complex with binding partners, suggesting that conformational adaptation or selection may be a critical aspect of IDP function, in analogy to the observed importance of dynamic structural rearrangements for enzymology. As such, there is significant interest in documenting the binding mechanism for IDPs and addressing whether they fold prior to, during, or after formation of an initial complex with their binding partners [4]. Pre-existing structure or "folding knowledge" encoded in the unbound state may provide an important reduction to the entropic penalty associated with folding-upon-binding; or it may be the case that the transient structures encountered in the unbound state are not important at all if multiple bound conformations are possible [5].

Here we have shown that apo-ctFCP1 has a tendency to adopt helical conformations in its RAP74 binding region, which will form a well ordered α-helix upon stable complex formation. To the best of our knowledge, this region of ctFCP1only interacts with binding partners in a helical conformation, making this tendency relevant to the folding-upon-binding mechanism. Still, the weak CD signal and only minimal bias towards α-helical conformation detected by NMR suggest that the sampling of helical space is temporally transient, non-cooperative, or both. There are multiple ensembles consistent with the spectroscopic signatures for α-helicity, many of which are not cooperative in nature and which do not satisfy

well our physicochemical intuition regarding the nature of the α-helix [27]. In fact, differential scanning calorimetry studies performed by the authors do not show a cooperative transition even in the presence of high TFE concentrations (data not shown), suggesting that the tendency toward helical structure should not be thought of in the same way as the nucleated two-state transitions seen in folding studies of globular proteins. The HeLa extract experiment shows the conformational tendencies reported are likely to be relevant to the state encountered by the RNAPII holoenzyme in the cell. Future studies aimed at fully defining the thermodynamics and kinetics of folding-upon-binding in this system will therefore be critical.

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References

[1] A.K. Dunker, C.J. Oldfield, J. Meng, P. Romero, J.Y. Yang, J.W. Chen, V. Vacic, Z. Obradovic, V.N. Uversky, The unfoldomics decade: an update on intrinsically disordered proteins, BMC Genomics 9 (Suppl. 2) (2008) S1.
[2] M. Fuxreiter, P. Tompa, I. Simon, V.N. Uversky, J.C. Hansen, F.J. Asturias, Malleable machines take shape in eukaryotic transcriptional regulation, Nat. Chem. Biol. 4 (2008) 728–737.
[3] K. Sugase, H.J. Dyson, P.E. Wright, Mechanism of coupled folding and binding of an intrinsically disordered protein, Nature 447 (2007) 1021–1025.
[4] P. Csermely, R. Palotai, R. Nussinov, Induced fit, conformational selection and independent dynamic segments: an extended view of binding events, Trends Biochem. Sci. 35 (2010) 539–546.
[5] V.N. Uversky, Seven lessons from one IDP structural analysis, Structure 18 (2010) 1069–1071.
[6] A. Meinhart, T. Kamenski, S. Hoeppner, S. Baumli, P. Cramer, A structural perspective of CTD function, Genes Dev. 19 (2005) 1401–1415.
[7] J. Archambault, G.H. Pan, G.K. Dahmus, M. Cartier, N. Marshall, S. Zhang, M.E. Dahmus, J. Greenblatt, FCP1, the RAP74-interacting subunit of a human protein phosphatase that dephosphorylates the carboxyl-terminal domain of RNA polymerase IIO, J. Biol. Chem. 273 (1998) 27593–27601.
[8] K. Kamada, R.G. Roeder, S.K. Burley, Molecular mechanism of recruitment of TFIIF-associating RNA polymerase C-terminal domain phosphatase (FCP1) by transcription factor IIF, Proc. Natl. Acad. Sci. USA 100 (2003) 2296–2299.
[9] B.D. Nguyen, K.L. Abbott, K. Potempa, M.S. Kobort, J. Archambault, J. Greenblatt, P. Legault, J.G. Omichinski, NMR structure of a complex containing the TFIIF subunit RAP74 and the RNA polymerase II carboxyl-terminal domain phosphatase FCP1, Proc. Natl. Acad. Sci. USA 100 (2003) 5688–5693.
[10] H.J. Dyson, P.E. Wright, Unfolded proteins and protein folding studied by NMR, Chem. Rev. 104 (2004) 3607–3622.
[11] M.R. Jensen, L. Salmon, G. Nodet, M. Blackledge, Defining conformational ensembles of intrinsically disordered and partially folded proteins directly from chemical shifts, J. Am. Chem. Soc. 132 (2010) 1270–1272.
[12] D.W. Bolen, I.V. Baskakov, The osmophobic effect: natural selection of a thermodynamic force in protein folding, J. Mol. Biol. 310 (2001) 955–963.
[13] H.X. Zhou, G. Rivas, A.P. Minton, Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences, Annu. Rev. Biophys. 37 (2008) 375–397.
[14] Y.C. Chang, T.G. Oas, Osmolyte-induced folding of an intrinsically disordered protein: folding mechanism in the absence of ligand, Biochemistry 49 (2010) 5086–5096.
[15] M. Wells, H. Tidow, T.J. Rutherford, P. Markwick, M.R. Jensen, E. Mylonas, D.I. Svergun, M. Blackledge, A.R. Fersht, Structure of tumor suppressor p53 and its intrinsically disordered N-terminal transactivation domain, Proc. Natl. Acad. Sci. USA 105 (2008) 5762–5767.
[16] T. Mittag, J. Marsh, A. Grishaev, S. Orlicky, H. Lin, F. Sicheri, M. Tyers, J.D. Forman-Kay, Structure/function implications in a dynamic complex of the intrinsically disordered Sic1 with the Cdc4 subunit of an SCF ubiquitin ligase, Structure 18 (2010) 494–506.
[17] T. Mittag, J.D. Forman-Kay, Atomic-level characterization of disordered protein ensembles, Curr. Opin. Struct. Biol. 17 (2007) 3–14.
[18] M.R. Jensen, P.R. Markwick, S. Meier, C. Griesinger, M. Zweckstetter, S. Grzesiek, P. Bernado, M. Blackledge, Quantitative determination of the conformational properties of partially folded and intrinsically disordered proteins using NMR dipolar couplings, Structure 17 (2009) 1169–1185.
[19] W. Bermel, I. Bertini, I.C. Felli, M. Piccioli, R. Pierattelli, ^{13}C-detected protonless NMR spectroscopy of proteins in solution, Prog. Nucl. Magn. Reson. Spectrosc. 48 (2006) 25–45.
[20] B. O'Hare, A.J. Benesi, S.A. Showalter, Incorporating^{1}H-chemical shift determination into^{13}C-direct detected spectroscopy of intrinsically disordered proteins in solution, J. Magn. Reson. 200 (2009) 354–358.
[21] S.A. Showalter, NMR assignment of the intrinsically disordered C-terminal region of Homo sapiens FCP1 in the unbound state, Biomol. NMR Assign. 3 (2009) 179–181.
[22] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, Nmrpipe – a multidimensional spectral processing system based on Unix pipes, J. Biomol. NMR 6 (1995) 277–293.
[23] T. Goddard, D. Kneller, SPARKY 3.113, 2006.
[24] D.S. Wishart, B.D. Sykes, The C-13 chemical-shift index – a simple method for the identification of protein secondary structure using C-13 chemical-shift data, J. Biomol. NMR 4 (1994) 171–180.
[25] B. Meszaros, I. Simon, Z. Dosztanyi, Prediction of protein binding regions in disordered proteins, PLoS Comput. Biol. 5 (2009) e1000376.
[26] M. Jiao, H.T. Li, J. Chen, A.P. Minton, Y. Liang, Attractive protein–polymer interactions markedly alter the effect of macromolecular crowding on protein association equilibria, Biophys. J. 99 (2010) 914–923.
[27] E. Johnson, S.A. Showalter, R. Brüschweiler, A multifaceted approach to the interpretation of NMR order parameters: a case study of a dynamic alpha-helix, J. Phys. Chem. B 112 (2008) 6203–6210.
[28] Z. Dosztanyi, B. Meszaros, I. Simon, ANCHOR: web server for predicting protein binding regions in disordered proteins, Bioinformatics 25 (2009) 2745–2746.