Induced folding of the U2AF35 RRM upon binding to U2AF65

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Abstract The human essential splicing factor U2AF (U2 auxiliary factor) consists of 35 and 65 kDa subunits which form a highly stable heterodimer in solution. Copurification of the recombinant U2AF35 RNA recognition motif (U2AF35 RRM) and full-length U2AF65 yields a soluble and functionally active minimal U2AF heterodimer. Recombinant U2AF35 RRM protein free and in complex with three different regions of U2AF65 was characterized by nuclear magnetic resonance spectroscopy. We found that the recombinant U2AF35 RRM is unstructured in solution but its tertiary structure is induced upon binding to U2AF65. This interaction is mediated by the N-terminal proline-rich region of U2AF65 and does not involve the U2AF65 RRMs.

Key words: U2 auxiliary factor; RNA recognition motif; Folding; Nuclear magnetic resonance; Splicing

1. Introduction

In eukaryotic cells, the intervening non-coding sequences (introns) have to be removed from the mRNA precursors. This process, called splicing, is initiated with the recognition of intron defining sequences by the splicing machinery. The U2 auxiliary factor (U2AF) plays a critical role in the selection of the intron 3′-splice site, which is composed of a polypyrimidine tract and an AG dinucleotide. U2AF is a heterodimer and consists of a large (65 kDa) and a small (35 kDa) subunit in humans [1]. The large subunit (U2AF65) contains an N-terminal arginine/serine (RS)-rich domain, a proline-rich region (P) and three RNA recognition motifs (RRM) (Fig. 1). The primary structure of the small subunit (U2AF35) comprises four domains (Fig. 1): a central RRM that is flanked by two CCCH zinc-binding domains (Zn) and a C-terminal RS domain.

Both subunits interact with the pre-mRNA. U2AF65 specifically recognizes the polypyrimidine tract via its RRMs [2], whereas U2AF35 contacts the conserved AG [3–5]. Due to the weak homology between the U2AF35 RRM and classical RRM-type RNA-binding domains, the function of the U2AF35 RRM remained unclear until recently. In a previous study, it was shown that the U2AF35 RRM is sufficient to activate splicing of the mouse immunoglobulin M pre-mRNA [6]. This activity depends on the ability of the U2AF35 RRM to bind both U2AF65 and the pre-mRNA.

Recently, the crystal structure of a core U2AF heterodimer, comprising the U2AF35 RRM bound to the U2AF65 P-rich region, was described showing the structural basis for the formation of a minimal U2AF65/35 heterodimer [7]. In an attempt to characterize the U2AF heterodimer interaction in solution, we cloned, overexpressed and purified several complexes between the U2AF35 RRM and various regions of U2AF65. We used nuclear magnetic resonance (NMR) to examine conformational changes that occur in U2AF35 RRM upon U2AF65 binding. Our data show that the recombinant U2AF35 RRM alone is largely unstructured in solution, but adopts a tertiary structure upon binding to a U2AF65 fragment which contains the U2AF65 RRM1 in addition to the P-rich region.

2. Materials and methods

2.1. Cloning

U2AF65(1–475) cloning was previously described [6]. DNA encoding U2AF35(1–240), U2AF35(1–187), U2AF35(1–180), U2AF35(38–152), U2AF65(88–237), U2AF65(98–237) and U2AF65(88–342) was PCR amplified from the full-length cDNA of the proteins with specific primers. All 5′-sense primers include an *Nco*I restriction site which adds an extra methionine to the N-terminus of the expressed protein products. All 3′-reverse primers contain a *Kpn*I site and a stop codon. Digested PCR fragments were inserted into pET-based expression vectors as summarized in Table 1. Plasmids (pACYCT7) for coexpression experiments are based on standard pACYC177 (Biolabs) low copy number plasmids with transferred T7 expression cassette from pET vectors (Novagen) (G. Stier, unpublished results).

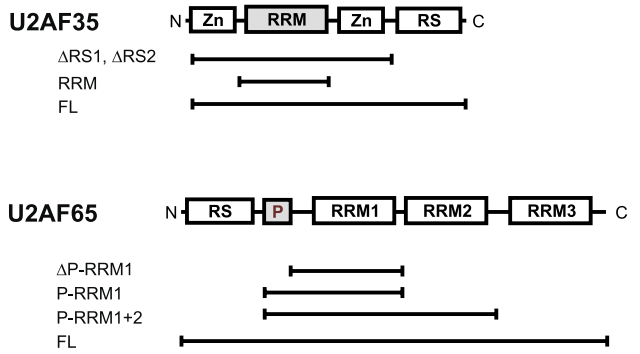

Fig. 1. U2AF domains. Schematic depiction of protein constructs expressed. Abbreviations are explained in text.

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Abbreviations: U2AF, U2 auxiliary factor; RRM, RNA recognition motif; NMR, nuclear magnetic resonance; mRNA, messenger RNA

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2.2. Expression and purification

U2AF35 FL (full-length), ΔRS1 (deleted RS domain 1), ΔRS2 and RRM were either expressed separately with an N-terminal six-histidine (N-His) tag or coexpressed with untagged U2AF65 FL (Table 1, Fig. 1). Untagged U2AF65 ΔP-RRM1 (linker domain missing the P-rich region) was coexpressed with N-His-tagged U2AF35 RRM. Untagged U2AF65FL, N-His-tagged U2AF65 P-RRM1 and P-RRM1+2 proteins were expressed separately.

Plasmids were transfected into *Escherichia coli* BL21(DE3). BL21(DE3) pLysS was used for the expression of U2AF35 proteins. Transformed cells were plated onto Luria–Bertani (LB) agarose plates containing the required antibiotics (25 μg/ml kanamycin and/or 100 μg/ml ampicillin) and incubated over night at 37°C. For U2AF35 constructs, large pLysS colonies were selected and restreaked on fresh LB agarose plate containing 25 μg/ml kanamycin and 50 μg/ml chloramphenicol. Single colonies were picked and grown overnight at 37°C in liquid medium containing the required antibiotics. The preculture was then diluted 50 times in fresh medium at 37°C. Unlabeled and ^{15}N-labeled proteins were produced using LB, and standard M9 medium with ^{15}N-NH_4Cl as sole nitrogen source [8], respectively. The cells were induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at $OD_{600\,nm}$ of 0.7–0.8. U2AF65 FL, P-RRM1 and P-RRM1+2 domains were expressed at 37°C for 3 h, all U2AF35 domains (including those coexpressed with U2AF65 FL or ΔP-RRM1) were expressed at 18°C for 16 h.

Cells grown in 1 l of culture were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris–HCl, 0.2–0.7 M NaCl, 5 mM β-mercaptoethanol, 10% glycerol, 0.1% Triton, 0.1 mg/ml lysozyme, 1 mM Pefabloc, pH 7.5), disrupted by sonication and centrifuged for 1 h at 4°C at 100 000 × *g*.

All soluble recombinant proteins and complexes were purified by affinity chromatography (Ni-NTA agarose, Qiagen) followed by a gel filtration (Superdex 75 or 200 16/60, Pharmacia). Ni-NTA columns were equilibrated in 50 mM Tris–HCl, 200 mM NaCl, 5 mM β-mercaptoethanol and 20 mM imidazole (pH 8), and bound proteins were eluted by increasing the concentration of imidazole to 300 mM. Gel filtration runs were performed in 20 mM sodium phosphate, 100 mM NaCl, 1 mM dithiothreitol and 0.02% NaN_3 (pH 6), at 1 ml/min flow rate.

For U2AF65 P-RRM1 and P-RRM1+2 proteins, the N-His tag was removed after the first Ni-NTA column using recombinant histidine-tagged tobacco etchvirus (TEV) protease, leaving two additional residues (glycine–alanine) at the N-terminus of the protein prior to the methionine. A second Ni-NTA column eliminates both the protease and the uncleaved proteins.

U2AF35 RRM expression and purification yielded ~5 mg and ~1 mg of protein from 1 l LB and minimal media, respectively. The expression yields for the different U2AF65 domains are about twice more. To obtain U2AF65 P-RRM1/U2AF35 RRM and U2AF65 P-RRM1+2/U2AF35 RRM complexes, the individual proteins were mixed in equimolar ratio, as judged from the Coomassie staining of the pure proteins on SDS–polyacrylamide gel electrophoresis (Fig. 2A). Complexes formed were further purified using gel filtration (Fig. 2B).

Protein concentrations, determined by absorbance measurement at 280 nm using theoretical extinction coefficients, range from 0.1 to 0.5 mM. NMR spectra were measured in a 20 mM sodium phosphate (pH 6.0–6.8) buffer in H_2O/D_2O 9:1, containing 50 mM NaCl, 1 mM dithiothreitol and 0.02% NaN_3.

2.3. NMR spectroscopy

NMR spectra were collected on a Bruker DRX600 spectrometer at 295 or 303 K. Two dimensional ^{1}H,^{15}N HSQC (heteronuclear single quantum correlation) [9,10] or TROSY (transverse relaxation-optimized spectroscopy) [11,12] experiments were recorded on the free ^{15}N-labeled U2AF35 RRM, and on the U2AF65 P-RRM1/U2AF35 RRM and U2AF65 P-RRM1+2/U2AF35 RRM complexes, which were ^{15}N-labeled either for the U2AF35 or the U2AF65 subunit.

3. Results and discussion

3.1. The U2AF35 RRM domain is expressed soluble

We designed and cloned four different U2AF35 proteins fragments: the FL protein, two constructs lacking the C-terminal RS domain (ΔRS1 and ΔRS2) and the RRM alone. All U2AF35 proteins were toxic when overexpressed in *E. coli*, as indicated by a slow growth rate of transformed BL21(DE3) in the liquid culture. Toxicity was reduced when using a BL21(DE3) pLysS strain. After cell lysis, U2AF35 FL, ΔRS1 and ΔRS2 were localized in inclusion bodies. Only U2AF35 RRM was expressed soluble when bacteria were grown at a temperature below 25°C. The solubility properties of the U2AF35 constructs were not improved upon coexpression of the U2AF35 FL. Presumably, this results from the instability of expression plasmids even when using a two-plasmid coexpression system with plasmids of different origin and antibiotic resistance (see Table 1), as has been previously observed [13].

3.2. The free U2AF35 RRM is unstructured in solution

The soluble ^{15}N-labeled U2AF35 RRM was purified using Ni-NTA affinity chromatography followed by gel filtration. The gel filtration profile exhibits a single symmetrical peak with an elution volume corresponding to a ~16 kDa protein (Fig. 2B). While the protein appears to be monomeric this unexpected elution volume is not indicative of a globular protein. We therefore used NMR spectroscopy to determine whether the recombinant protein is properly folded in solution. The chemical shift dispersion and line widths in ^{1}H and ^{15}N NMR spectra allow to distinguish whether a protein is

Table 1
Cloning of U2AF65/U2AF35 domains

Domain name	Residue range	MW (kDa)	Source vector	Tag	Selection
U2AF35					
FL	1–240	27.9	pET9-d	N-His	kanamycin
			pACYCT7	–	kanamycin
ΔRS1	1–187	21.7	pET24-d	N-His-TEV	kanamycin
			pACYCT7	–	kanamycin
ΔRS2	1–180	20.7	pET9-d	N-His	kanamycin
			pACYCT7	–	kanamycin
RRM	38–152	13.3	pET9-d	N-His	kanamycin
			pACYCT7	–	kanamycin
			pET24-d	N-His-GST-TEV	kanamycin
U2AF65					
FL	1–475	53.5	pET21-d(+)	–	ampicillin
ΔP-RRM1	98–237	15.3	pET21-d(+)	–	ampicillin
P-RRM1	88–237	16.6	pET24-d	N-His-TEV	kanamycin
P-RRM1+2	88–342	27.7	pET24-d	N-His-TEV	kanamycin

In all pET24-d derivatives, a sequence coding for a tobacco etchvirus (TEV) protease cleavage site is localized after the tag.

completely unfolded (i.e. adopting a random coil conformation), misfolded (or molten-globule like) or folded [14]. Fig. 3A shows the $^{1}H,^{15}N$ HSQC spectrum of the ^{15}N-labeled U2AF35 RRM. The small chemical shift dispersion clearly indicates that the protein adopts a random coil conformation and is unstructured in aqueous solution.

These findings also show that the soluble expression of the U2AF35 RRM does not imply that the protein adopts a tertiary structure. Note, that the U2AF35 RRM protein expressed as a fusion with a glutathione *S*-transferase (GST) shows the same behavior based on a $^{1}H,^{15}N$ HSQC spectrum of the free U2AF35 RRM obtained after GST proteolytic cleavage. Thus, the presence of the GST carrier protein during expression is not able to induce a structured protein. Similar observations have been previously described in the literature [15,16]. The expression result most likely reflects the intrinsic propensity of the unbound protein to be unstructured yet not prone to aggregation.

3.3. U2AF35 RRM complex with U2AF65 FL

The soluble U2AF35 RRM was used to prepare a recombinant heterodimeric complex with U2AF65 FL [6]. Briefly, the His-tagged U2AF35 RRM was immobilized on a Ni-NTA column, and the resin was incubated with the clear lysate of *E. coli* cells expressing untagged U2AF65 FL. After extensive washing of the column, the bound U2AF65 FL/U2AF35 RRM complex was eluted with imidazole and purified on a Superdex 200 gel filtration column. It has been recently shown, that this recombinant heterodimeric U2AF complex is fully active in in vitro splicing assays. Indeed, U2AF65 FL/U2AF35 RRM restores splicing in U2AF-depleted HeLa nuclear extract [6]. This functional activity suggests that within the U2AF heterodimer the U2AF35 RRM adopts a native conformation, which is induced upon binding to U2AF65 in vitro.

However, the elution volume of the complex during gel filtration shows an apparent molecular weight (MW) above 400 kDa. Furthermore, a $^{1}H,^{15}N$ HSQC spectrum recorded on a 0.1 mM sample of a ^{15}N-labeled U2AF65 FL/unlabeled U2AF35 RRM complex (data not shown) is consistent with the high MW of an aggregated state since most of the backbone resonances of U2AF35 RRM show severe line-broadening or are not observable. It is conceivable that at the lower concentration used for the in vitro splicing assays a fraction of the complex is not aggregated and thus confers the functional activity. In contrast, the high concentrations used for gel filtration and NMR might be above the concentration limit for aggregation of the U2AF heterodimer.

3.4. U2AF35 RRM complexes with smaller regions of U2AF65

The U2AF35 interacting region of U2AF65 has been previously mapped to the P-rich fragment spanning residues K90 to Y107 [7,13] in the linker between the N-terminal RS domain and RRM1 (Fig. 1, region 'P'). We have cloned and expressed three constructs of U2AF65 comprising a truncated linker lacking the P-rich region followed by the first RRM (ΔP-RRM1, residues 98–237), the linker followed by the first RRM (P-RRM1, residues 88–237), and the linker followed by the first and the second RRM (P-RRM1+2, residues 88–342). Pairwise interactions between N-His-tagged U2AF35 RRM and the different U2AF65 untagged constructs were probed using affinity chromatography [17]. As expected, U2AF65 ΔP-RRM1 was not able to form a stable heterodimeric complex with U2AF35 RRM. On the other hand, both U2AF65 P-RRM1 and P-RRM1+2 were retained on a Ni-NTA affinity column via interaction with U2AF35 RRM.

Therefore, U2AF65 P-RRM1/U2AF35 RRM and U2AF65 P-RRM1+2/U2AF35 RRM complexes were prepared by co-purification of U2AF35 RRM with U2AF65 P-RRM1 and P-RRM1+2, which were expressed separately as N-His tag

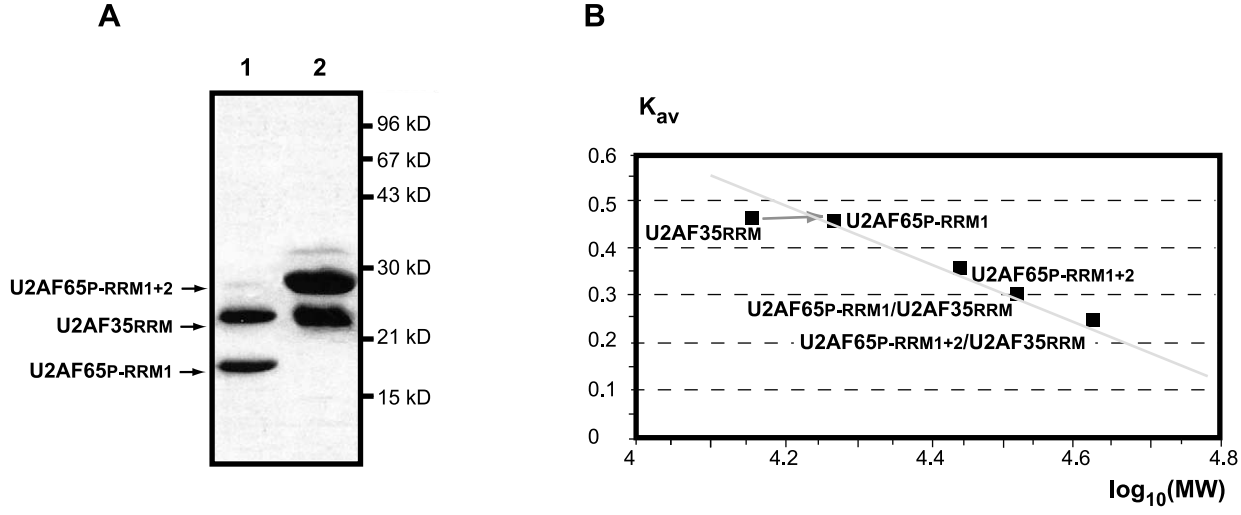

Fig. 2. Biochemical data for the U2AF complexes. A: SDS–polyacrylamide gel electrophoresis of U2AF65 P-RRM1/U2AF35 RRM (lane 1) and U2AF65 P-RRM1+2/U2AF35 RRM (lane 2) complexes. The abnormal migration of the U2AF35 RRM presumably results from the unusual distribution of charged amino acids [27]. B: Gel filtration on a Superdex 75 HiLoad 16/60 of recombinant proteins and complexes: K_{av} are plotted as a function of the calculated MW logarithm ($\log_{10}$MW). $K_{av} = (V_S - V_0)/(V_C - V_0)$ where V_S, V_0 and V_C are the sample elution, void and column volumes, respectively. The depicted selectivity curve (gray line) indicates theoretical K_{av} values within the fractionation range of the column. In contrast to U2AF65 P-RRM1 and U2AF65 P-RRM1+2, free or in complex with U2AF35 RRM, the apparent MW of free U2AF35 RRM is 1.2-fold larger than the theoretical MW (indicated by arrows).

fusion proteins. After Ni affinity purification and proteolytic cleavage of the N-His tag, the U2AF65 fragments were mixed with purified N-His-tagged U2AF35 RRM. During gel filtration single symmetrical peaks are observed indicating homogeneity of the two complexes. The elution volumes (Fig. 2B) are in good agreement with the MWs expected for the heterodimers. The separate expression of U2AF35 and U2AF65 proteins and successive in vitro complex formation allows to prepare subunit-selective isotopically labelled heterodimers for NMR studies where this is beneficial to reduce spectral overlap.

3.5. Induced folding of U2AF35 RRM upon binding to U2AF65

The conformation of U2AF35 RRM when bound to each of the two U2AF65 fragments comprising the P-rich region (U2AF65 P-RRM1, P-RRM1+2) was monitored by NMR. $^{1}H,^{15}N$ HSQC experiments recorded on complexes formed with ^{15}N-labeled U2AF35 RRM and unlabeled U2AF65 P-RRM1 or U2AF65 P-RRM1+2 clearly show that the U2AF35 RRM is folded (Fig. 3B). Furthermore, the NMR spectra of U2AF35 RRM in the two heterodimers are superimposable. Thus, the chemical shifts of U2AF35 RRM are not perturbed by addition of the second RRM of U2AF65 indicating that it does not contact the U2AF35 RRM.

3.6. Contribution of U2AF65 for complex formation with U2AF35 RRM

We also recorded $^{1}H,^{15}N$ HSQC experiments on ^{15}N-labeled U2AF65 P-RRM1/U2AF35 RRM and ^{15}N-labeled U2AF65 P-RRM1+2/U2AF35 RRM complexes, which were only labelled in the U2AF65 subunit. The spectra shown in Fig. 3C confirm that in both complexes the U2AF65 RRM domains adopt a tertiary structure as expected. The HSQC spectra also resemble those reported for the separate U2AF65 RRM1 and RRM2 domains [18]. This suggests that there are no large structural rearrangements involving the U2AF65 RRM1 and RRM2 domains upon binding to U2AF35 RRM. These findings are consistent with the recently determined crystal structure of a minimal U2AF heterodimer, which shows that the P-rich region of U2AF65 is necessary and sufficient to mediate the interaction with U2AF35 RRM [7]. The crystal structure of a minimal U2AF heterodimer (U2AF35 RRM/U2AF65 P) revealed that the U2AF35 RRM when bound to the P-rich region of U2AF65 adopts a canonical RRM-fold comprising a four-stranded anti-parallel β-sheet packed against two α-helices (Fig. 4B). Structure-based alignment of the U2AF35 RRM sequence with other RRMs (Fig. 4A) shows that hydrophobic residues that form the structural core of the RRM fold are all present in

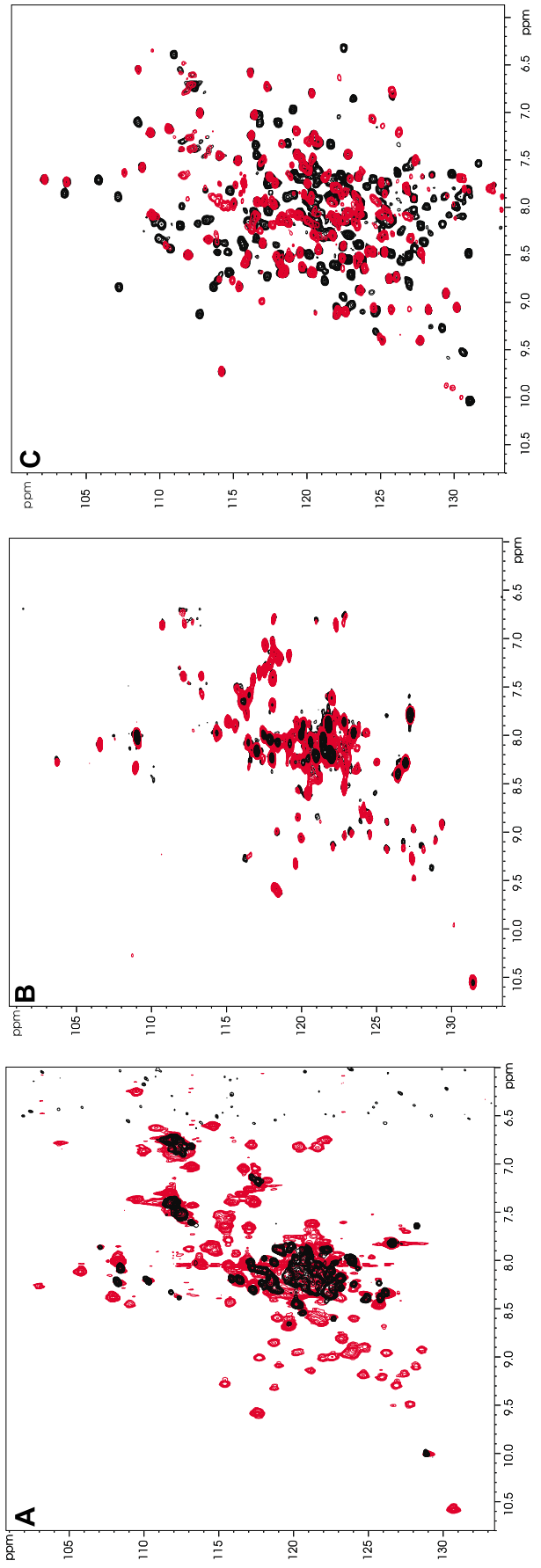

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Fig. 3. NMR spectra of U2AF complexes. A: Superimposition of $^{1}H,^{15}N$ HSQC spectra of ^{15}N-labeled U2AF35 RRM free (black) and when bound to unlabeled U2AF65 P-RRM1 (red) at 295 K. B: Superimposition of $^{1}H,^{15}N$ TROSY spectra of U2AF heterodimer complexes consisting of ^{15}N-labeled U2AF35 RRM bound to U2AF65 P-RRM1 (black) or U2AF65 P-RRM1+2 (red) at 303 K. The similarity of the two spectra indicates that the interface of U2AF35 RRM with U2AF65 is virtually identical in the two complexes. C: $^{1}H,^{15}N$ TROSY spectra of ^{15}N-labeled U2AF65 P-RRM1/unlabeled U2AF35 RRM (red) and ^{15}N-labeled U2AF65 P-RRM1+2/unlabeled U2AF35 RRM (black) at 295K. Most signals of the U2AF65 P-RRM1 coincide with corresponding signals in the larger construct U2AF65 P-RRM1+2 that comprises both RRMs.

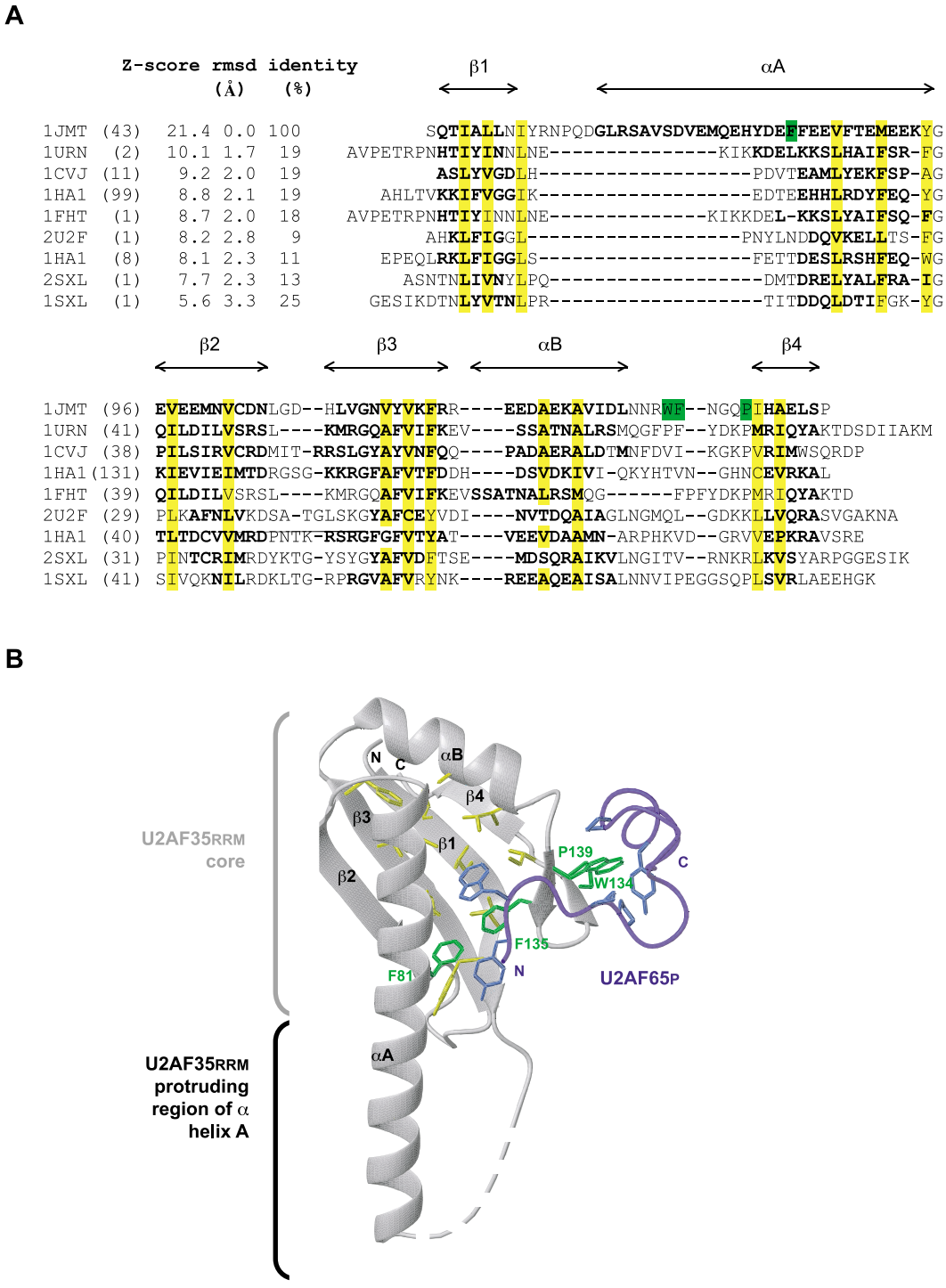

Fig. 4. U2AF35 RRM sequence–structure relationship. A: Structure-based alignment of the U2AF35 RRM sequence (PDB accession number: 1jmt) with sequences of canonical RRMs (1urn: human heterogeneous nuclear ribonucleoprotein A1, 1cvj: human polyadenylate-binding protein 1, 1ha1: human heterogeneous nuclear ribonucleoprotein A1, 1fht: human U1 small nuclear ribonucleoprotein A, 2u2f: human U2AF65, 2sxl and 1sxl: *Drosophila* sex lethal protein). The number of the first residue is given following PDB code. Pairwise comparisons of structures were performed between U2AF35 RRM and all the other RRMs using DALI server (http://www.ebi.ac.uk/dali/); structural similarity is quantified by the *Z*-score, the root mean square deviation between equivalent atoms and the percentage of sequence identity. Secondary structure elements are indicated on the top of the alignment. Their precise boundaries in the different proteins are indicated by bold print. Amino acids colored in yellow are conserved hydrophobic residues that form the structural core. U2AF35 RRM amino acids colored in green are the solvent accessible hydrophobic and aromatic residues that become buried in the U2AF35 RRM/U2AF65 P complex [7]. B: Ribbon representation of the U2AF35 RRM/U2AF65 P complex [7]. U2AF35 RRM and U2AF65 P are colored in gray and blue, respectively. Side chains of U2AF35 RRM highlighted in the alignment (yellow, green) as well as side chains of U2AF65 P residues that mediate the U2AF35 RRM interaction (blue) are displayed.

U2AF35. However, the U2AF35 RRM fold also exhibits three distinctive features. First, a 20 amino acid long sequence insertion in U2AF35 RRM leads to an unusually long protruding helix A, preceded by a disordered loop. Secondly, the U2AF35 RRM contains numerous negatively charged residues, many of which are located in this elongated helix A. Thirdly, the U2AF35 RRM exhibits a solvent-exposed hydrophobic patch (including F81, W134, F135 and P139) which mediates the U2AF65 interaction. In total, 26% of the solvent accessible surface of U2AF35 RRM is buried in the U2AF minimal complex [7] such that the heterodimer interface may be considered as an additional intermolecular hydrophobic core. These sequence characteristics of the U2AF35 RRM may thus play a role for our finding that the recombinant free U2AF35 RRM is unstructured in solution. Alternatively, it is also possible that the Zn knuckles flanking the RRM domain N- and C-terminally in the U2AF35 FL protein contribute to stabilizing the tertiary structure of the RRM domain even in the absence of the U2AF65 ligand. Further experiments are required to distinguish between these possibilities. In any case, folding of the U2AF35 RRM as shown in the NMR spectra is likely induced upon binding to the U2AF65 P-rich region. This is demonstrated by the crystal structure of the U2AF35 RRM bound to the P-rich region of U2AF65 and further supported by the similarity of NMR spectra of the U2AF65 RRMs free and bound to U2AF35 RRM (see above). Therefore, it appears that different regions of U2AF65 are used in a modular way to mediate contacts with U2AF35 (the P-rich region) and the pre-mRNA (RRM1+2), respectively [19].

4. Conclusion

It is well known that an unstructured protein may undergo an induced transition from random coil to a well-defined conformation upon interaction with a binding partner which may be a nucleic acid [20,21] or a protein [22–24]. Two unstructured domains can also mutually act as chaperones towards each other [25,26]. In the present paper, we found that the monomeric free form of U2AF35 RRM is unstructured in solution and that its three-dimensional structure is induced upon binding to its native protein partner U2AF65.

The characterization of the U2AF35 RRM when bound to different regions of U2AF65 is a prerequisite for future structural and functional studies of the U2AF heterodimer and its interaction with the pre-mRNA during spliceosome assembly. Supplementary material (U2AF35 sequence alignment) can be found on the web at http://www.elsevier.com/PII/S0014579302032945.

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References

[1] Zamore, P. and Green, M. (1989) Proc. Natl. Acad. Sci. USA 86, 9243–9247.
[2] Zamore, P., Patton, J. and Green, M. (1992) Nature 355, 609–614.
[3] Zorio, D. and Blumenthal, T. (1999) Nature 402, 835–838.
[4] Merendino, L., Guth, S., Bilbao, D., Martinez, C. and Valcárcel, J. (1999) Nature 402, 838–841.
[5] Wu, S., Romfo, C., Nilsen, T. and Green, M.R. (1999) Nature 402, 832–835.
[6] Guth, S., Tange, T., Kellenberger, E. and Valcárcel, J. (2001) Mol. Cell. Biol. 21, 7673–7681.
[7] Kielkopf, C.L., Rodionova, N.A., Green, M.R. and Burley, S.K. (2001) Cell 106, 595–605.
[8] Sambrook, J., Fritsch, E. and Maniatis, T. (1989), Molecular Cloning: A Laboratory Manual, Vol. 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[9] Kay, L., Kiefer, P. and Saarinen, T. (1992) J. Am. Chem. Soc. 114, 10663–10665.
[10] Sattler, M., Schleucher, J. and Griesinger, C. (1999) Prog. NMR Spectrosc. 34, 93–158.
[11] Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) Proc. Natl. Acad. Sci. USA 94, 12366–12371.
[12] Salzmann, M., Wider, G., Pervushin, K. and Wüthrich, K. (1999) J. Biomol. NMR 15, 181–184.
[13] Rudner, D.Z., Kanaar, R., Breger, K.S. and Rio, D.C. (1998) Mol. Cell. Biol. 18, 1765–1773.
[14] Yao, J., Dyson, H.J. and Wright, P.E. (1997) FEBS Lett. 419, 285–289.
[15] Nomine, Y., Ristriani, T., Laurent, C., Lefevre, J., Weiss, E. and Trave, G. (2001) Protein Expr. Purif. 23, 22–32.
[16] Sachdev, D. and Chirgwin, J. (1999) J. Protein Chem. 18, 127–136.
[17] Fribourg, S., Romier, C., Werten, S., Gangloff, Y., Poterszman, A. and Moras, D. (2001) J. Mol. Biol. 306, 363–373.
[18] Ito, T., Muto, Y., Green, M. and Yokoyama, S. (1999) EMBO J. 18, 4523–4534.
[19] Valcárcel, J., Gaur, R.K., Singh, R. and Green, M.R. (1996) Science 273, 1706–1709.
[20] Marmorstein, R., Carey, M., Ptashne, M. and Harrison, S. (1992) Nature 356, 408–414.
[21] Baleja, J., Marmorstein, R., Harrison, S. and Wagner, G. (1992) Nature 356, 450–453.
[22] Uesugi, M., Nyanguile, O., Lu, H., Levine, A. and Verdine, G. (1997) Science 277, 1310–1313.
[23] Atkinson, R., Joseph, C., Piaz, F.D., Birolo, L., Stier, G., Pucci, P. and Pastore, A. (2000) Biochemistry 39, 5255–5264.
[24] Warnmark, A., Wikstrom, A., Wright, A., Gustafsson, J. and Hard, T. (2001) J. Biol. Chem. 276, 45939–45944.
[25] Demarest, S.J., Martinez-Yamout, M., Chung, J., Chen, H., Xu, W., Dyson, H.J., Evans, R.M. and Wright, P.E. (2002) Nature 415, 549–553.
[26] Zhang, Q., Fan, J.S. and Zhang, M. (2001) J. Biol. Chem. 276, 43216–43220.
[27] Graceffa, P., Jancso, A. and Mabuchi, K. (1992) Arch. Biochem. Biophys. 297, 46–51.