Structures of intermediates during RES complex assembly

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The action of the spliceosome depends on the stepwise cooperative assembly and disassembly of its components. Very strong cooperativity was observed for the RES (Retention and Splicing) heterotrimeric complex where the affinity from binary to tertiary interactions changes more than 100-fold and affects RNA binding. The RES complex is involved in splicing regulation and retention of not properly spliced pre-mRNA with its three components—Snu17p, Pml1p and Bud13p—giving rise to the two possible intermediate dimeric complexes Pml1p-Snu17p and Bud13p-Snu17p. Here we determined the three-dimensional structure and dynamics of the Pml1p-Snu17p and Bud13p-Snu17p dimers using liquid state NMR. We demonstrate that localized as well as global changes occur along the RES trimer assembly pathway. The stepwise rigidification of the Snu17p structure following the binding of Pml1p and Bud13p provides a basis for the strong cooperative nature of RES complex assembly.

The key step of producing mature and nuclear export ready mRNA particles involves excision of introns in a process termed splicing. Responsible for the catalysis and orchestration of this process is the spliceosome, a multimegadalton assembly of proteins and snRNAs. Unlike ribosomes at the onset of translation, each complete and active spliceosome needs to assemble on its substrate de novo during the splicing cycle. Assembly, disassembly and remodelling of the spliceosome is therefore important. As part of this dynamic process various subcomplexes of changing composition are formed. In line with an efficient remodelling of the spliceosome, the spliceosomal proteins are believed not to act independently. Instead cooperative binding, resulting in a cooperative cascade, might drive spliceosome formation and thus its function.

One of the few protein complexes controlling both splicing and export of pre-mRNA is the retention and splicing (RES) complex. A set of introns, specifically associated with pre-mRNA of proteins engaged in nucleotide metabolism, shows splicing controlled by RES. The RES complex is composed of the 17.1 kDa small nuclear ribonucleoprotein-associated protein 17 (Snu17p), the pre-mRNA leakage protein 1 (Pml1p) and the 30.5 kDa bud site selection protein 13 (Bud13p). RES was shown to interact with U2 snRNA splicing factor 3B (U2 SF3B) proteins and Bud13p showed chemical crosslinks to human sap homolog 155 (Hsh155). In addition, we showed that Snu17p can be crosslinked to pre-mRNA between the branch point and 3′ splice site.

Recently, we determined the three-dimensional structure of the core of the RES trimer composed of Snu17p, Pml1p and Bud13p. We further demonstrated that the RES trimer assembles and forms a ternary complex with RNA in a highly cooperative manner. In order to obtain further insight into the molecular basis of the cooperative nature of RES assembly, we here present the three-dimensional structures of two dimeric intermediates along the RES assembly pathway. The structures of the two intermediates provide insight into the atomistic details of the rearrangements that are required in order to accommodate the two intrinsically disordered protein fragments of Bud13p and Pml1p and reveal the nature of the conformational plasticity of RES intermediates.

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Material and methods

Natural abundance and isotopically labelled Snu17p (residues 25–138 of Snu17p), Pml1p (residues 22–42 of Pml1p) and Bud13p (residues 215–255 of Bud13p) were prepared as described previously. Samples contained either 13C,15N-labelled peptide with natural abundance Snu17p or 13C,15N-labelled Snu17p with natural abundance peptide. In case of the Bud13p–Snu17p dimer, we also prepared a sample where both components of the dimer were 13C,15N labelled. For measurement of HN residual dipolar couplings (RDCs), samples were aligned by the addition of Pf1 phage (ASLA Biotech). NMR experiments were carried out at 35 °C on 600, 700, 800 and 900 MHz Bruker spectrometers. Spectrometers were equipped with cryogenically cooled HCN or room temperature HCN probes. In addition to the protein backbones, which were sequence-specifically assigned previously, the sequence-specific backbone resonance assignment of Bud13p and Pml1p in their dimeric complexes with Snu17p was performed using 3D HNCA, HNCOCA, HNCA/CB, HNCO and 15N-edited NOE-HSQC experiments. We also assigned the sidechains in the dimeric complexes with the help of 3D HcCH-TOCSY, 3D HBCBCGCDHD and 3D 13C-edited NOE-HSQC (both aromatic and aliphatic) experiments. Inter-molecular NOEs were extracted from 13C F1-edited/15N F3-filtered HSQC-NOESY spectra, from the standard Bruker pulse sequence library, which were recorded with a mixing time of 120 ms, recycle delay of 1 s and 32 scans per increment. A total of 256 points in the indirect 1H dimension and 32 in the indirect 13C dimensions were acquired. FIDs were processed with NMRPipe or Topspin (Bruker) and the resulting spectra were analysed using ccpnmr Analysis 2.2.1.

The BSD-IPAP-HSQC experiment was used to measure HN RDCs. A total of 91 RDCs were obtained for the Snu17p in the Pml1p–Snu17p dimer and 75 for C-terminally extended cSnu17p in the cPml1p–cSnu17p dimer. In addition, 53 RDCs of residues located in well-defined regions were used for validation of the Bud13p–Snu17p structure. In total, 87 RDCs were obtained in both complexes. For pairwise analysis of RDC sets, all 91 RDCs observed in the Pml1p–Snu17p dimer were compared against values observed in the RES trimer. Moreover, 79 (65 for the core RRM and 14 in the unfolded C-terminal α-helix) RDCs observed in the Bud13p–Snu17p dimer were compared against RES. Finally, 87 RDCs were compared between the Bud13p–Snu17p and Pml1p–Snu17p dimers. The software PALES was used for RDC analysis.

Structures were calculated in CYANA 3.023 and refined using Xplor-NIH 2.3.4. The maximum upper distance limit was set to 6.5 Å and the reference distance to 4.25 Å. Structure calculations were supplemented by dihedral angle restraints that were derived from backbone chemical shifts using TALOS-N. Only high-confidence values (labelled by TALOS-N as „strong“) were used. Eight cycles of structure calculation using CYANAs noesassig.py protocol were carried out. Intermolecular NOE contacts, which were extracted from filtered/edited NOE experiments, were treated separately to the automatic CYANA protocols and were manually refined in an iterative manner. H-bonds in α-helices and β-sheets were identified from the initial structural ensemble and confirmed by H-D exchange in combination with NOE patterns. The structure, which was closest to the mean, was used as representative of an ensemble.

Unless stated otherwise, statistics and structural comparisons were determined using the well-structured parts of the three RES components, that is residues 32–62/74–108 of Snu17p and residues 223–238 of Bud13p in the Bud13p which comprises residues 215–255 of Bud13p as Collinet et al. reported that residues 246–255 form an α-helix. Using a variety of multidimensional NMR experiments the sequence-specific assignment of the Pml1p–Snu17p dimer as well as the Bud13p–Snu17p dimer was achieved. Based on the assignment of 88.0% (Bud13p–Snu17p dimer) and 91.2% (Pml1p–Snu17p dimer) of all 1H proton resonances, we collected a large number of unambiguous intramolecular and intermolecular Snu17p–Bud13p and Snu17p–Pml1p NOE distance restraints (Table 1). They defined the structure of the Pml1p–Snu17p dimer and the Bud13p–Snu17p dimer at high resolution (Figs 1 and 2A,C). The final ensembles displayed RMSDs for all heavy atoms of 1.21 Å and 1.31 Å, respectively, with Ramachandran plot statistics of 90.3, 8.5, 1.0, 0.1%, 88.2, 11.7, 0.1, 0.0% (Pml1p–Snu17p dimer) for core, allowed, generous and disallowed regions, respectively (Table 1). The quality of the structures was further validated by RDCs (Fig. 3A). Notably, the molecular alignment of Snu17p in Pf1 phage differed strongly between the Bud13p–Snu17p dimer and the RES trimer, as well as between Bud13p–Snu17p and Pml1p–Snu17p dimer, consistent with a release of the C-terminal helix in the Bud13p–Snu17p dimer (Supplementary Fig. 1C–E). At the same time, the alignment was nearly identical between Pml1p–Snu17p dimer and the RES trimer, in agreement with the stabilization of the C-terminal helix of Snu17p in the two complexes.
The structure of the cPml1p–cSnu17p dimer and the hcBud13p–cSnu17p dimer retain the β1α1β2β3α2β4 topology of RRMs (Figs 1 and 2) and the domain characteristics of Snu17p seen in the structure of the cRES trimer. Despite the apparent similarity of the Snu17p and Bud13p complex structure to prototypical U2AF homology motif (UHM) and UHM ligand motif (ULM) interactions, the mode of interaction appears to be different. Whereas in classical ULM–UHM complexes a central tryptophan is positioned in a deep hydrophobic pocket provided by the RRM domain, tryptophan 232 of Bud13p is found in a shallow space approximately 11 Å away from the canonical site in Snu17p. This is the case for both the cRES trimer as well as the hcBud13p–cSnu17p dimer, despite the lack of steric obstruction provided by cPml1p in the latter case. The charge distribution over all three structures appears to be similar although, the C-terminal region of Snu17p, which only forms an α-helix in the cPml1p–cSnu17p dimer and the cRES trimer but not in the hcBud13p–cSnu17p dimer (Fig. 1A), is partially positively charged and might contribute to RNA binding. The overall similar charge distribution suggests that optimization of the electrostatic interaction might not be the major contributor to the cooperativity, which was observed for binding of cRES to RNA when compared to monomeric Snu17p and the two dimers.

Molecular motions in intermediate structures of the RES complex assembly pathway. In a recently solved structure of residues 25–113 of Snu17p in complex with residues 222–256 of Bud13p the C-terminal region of Snu17p, which forms an α-helix in the RES trimer and contributes to RNA

| NMR distance and dihedral constraints | cSnu17p (cPml1p) | cPml1p (cSnu17p) | cSnu17p (hcBud13p) | hcBud13p (cSnu17p) |
|---------------------------------------|-----------------|-----------------|-------------------|-------------------|
| Distance restraints*                  |                 |                 |                   |                   |
| Total NOE                             | 1814            | 216             | 1019              | 322               |
| Intra-residue                         | 467             | 91              | 244               | 109               |
| Inter-residue                         | 1347            | 125             | 775               | 213               |
| Sequential ([i–j] = 1)                | 520             | 96              | 186               | 115               |
| Non-sequential ([i–j] > 1)           | 827             | 29              | 589               | 98                |
| Hydrogen bonds                        | 50              | —               | 40                | —                 |
| Protein–protein intermolecular        | 228             | 228             | 77                | 77                |
| Total dihedral angle restraints       |                 |                 |                   |                   |
| Protein                               |                 |                 |                   |                   |
| φ                                     | 100             | 15              | 69                | 27                |
| ψ                                     | 100             | 15              | 69                | 27                |

Table 1. NMR and refinement statistics for the complexes. *Excluding intermolecular restraints. †Pairwise r.m.s. deviation was calculated among all refined structures over residues 32–62, 74–108 (cSnu17p) and 223–238 (hcBud13p) in the hcBud13p–cSnu17p dimer, and 32–126 (cSnu17p) and 26–39 (cPml1p) in the cPml1p–cSnu17p dimer.
binding\(^1\), was not present and therefore did not allow analysis of this functionally important region in the dimeric complex with Bud13p. Based on chemical shift and \(^{15}\)N spin relaxation data, we predicted that Snu17p residues beyond 115 would be unstable in the Bud13p–Snu17p dimer. In addition, the relative orientation of the C-terminal \(\alpha\)-helix of Bud13p (marked by a dashed ellipsoid) is flexible. Red, Snu17p; pink Bud13p. (B) Pml1p–Snu17p dimer. Shown are the backbones of the 20 lowest-energy structures of the NMR ensemble. Blue, Snu17p; cyan Pml1p.

**Figure 1. Three-dimensional structures of intermediates during ‘RES complex assembly.** (A) Bud13p–Snu17p dimer. 20 lowest-energy structures (backbone atoms only) are shown. Residues 116–131 of Snu17p, which fold into an \(\alpha\)-helix in the ‘RES trimer\(^1\), remain flexible in the Bud13p–Snu17p dimer. In addition, the relative orientation of the C-terminal \(\alpha\)-helix of Bud13p (marked by a dashed ellipsoid) is flexible. Red, Snu17p; pink Bud13p. (B) Pml1p–Snu17p dimer. Shown are the backbones of the 20 lowest-energy structures of the NMR ensemble. Blue, Snu17p; cyan Pml1p.
Figure 2. Comparison of the 3D structures of the RES core complex, the 'Pml1p–'Snu17p dimer and the 'Bud13p–'Snu17p dimer. (A) 'Bud13p (pink) in complex with 'Snu17p (red). (B) NMR ensemble of the RES core complex (PDB code: 2MKC15). Gray, 'Snu17p; black 'Bud13p and 'Pml1p. (C) Structure of 'Snu17p (blue) in complex with 'Pml1p (cyan). 20 lowest-energy structures (backbone atoms only) are shown. The L63 – F73 loop is encircled in gray and the C-terminal region of 'Snu17p, when folded into an α-helix, in black and the disordered C-terminal part of 'Pml1p, in red. (D) Detailed view of the L63–F73 loop of 'Snu17p (blue) and residues 205–210 of 'Pml1p (cyan) in the 'Pml1p–'Snu17p dimer (upper panel). The same loop is shown below for the 'Bud13p–'Snu17p dimer. Experimentally observed NOE contacts are represented with black lines. (E) Residues 106–115 of Snu17p in the 'Bud13p–'Snu17p dimer. Residues 106–115 are shown as an ensemble in light-blue and the rest of the 'Bud13p–'Snu17p dimer as a single structure in red. Experimental NOE contacts between Y109 (orange), P111 (green) and I61 are schematically indicated with dashed lines. (F) Regions of Snu17p, which are dynamic in the 'Bud13p–'Snu17p dimer, were mapped onto the 3D structure of the 'Pml1p–'Snu17p dimer. Residues 35, 37, 40, 43 – 46, 75, 98, 99, 101, 102, 107, 110, 112 of 'Snu17p were marked in red as they showed R\(_e\) values exceeding 10Hz in NMR relaxation measurements12 and/or experienced line broadening of 10Hz above the average value among the folded part of 'Snu17p in \(^{1}H-^{15}N\) HSQC experiments12. L63–F73 of 'Snu17p was also marked in black to highlight the sparse NOE network as shown in (D) and therefore higher RMSD values as presented in (A) and consistent with lower than average heteronuclear NOE values reported in10. Flexible N- and C-terminal residues (20–32 and 113–138) were excluded from this analysis. α-helix 3 of Snu17p, which is not formed in the 'Bud13p–'Snu17p dimer, is labelled and constitutes the most dynamic element in the 'Bud13p–'Snu17p dimer structure (as seen in (A)).
\(\beta\)-sheet provides a structural basis for the finding that the ability of the \(c\)Bud13p–\(c\)Snu17p dimer to bind to RNA was diminished but not abolished\(^{12}\).

Additional mobility in the \(hc\)Bud13p–\(c\)Snu17p dimer when compared to \(c\)RES was observed for the loop between L63 and F73 of \(c\)Snu17p, which samples a larger conformational space when \(c\)Pml1p is absent (Figs 1A and 2A,C) This can be tracked back to a lack of interactions between residues R64–E66 of \(c\)Snu17p and I26, I28 and D31 of \(c\)Pml1p as well as sparse intra-loop contacts (Fig. 2D). Altogether, it gives rise to an at least three times lower amount of NOE contacts when \(c\)Pml1p is absent (Fig. 2D).

Moreover, the L63–F73 loop of \(c\)Snu17p was reported to have lower than average heteronuclear NOE values pointing to increased pico-to-nanosecond motions\(^{10}\). Although the chemical exchange contribution to the \(R_2\) \(^{15}\)N relaxation rate \((R_{ex})\) in this region was not elevated, the adjacent loop (V40–E46) showed increased \(R_{ex}\) values when compared to the \(c\)Pml1p–\(c\)Snu17p dimer and the \(c\)RES trimer\(^{12}\). In addition, the \(\beta\)-turn adjacent to V40–E46 showed an elevated \(R_{ex}\) contribution and was affected by NMR line broadening\(^{12}\). The two loops and the adjacent \(\beta\)-turn are the site of \(c\)Pml1p binding, together with the C-terminal region of \(c\)Snu17p, which folds into an \(\alpha\)-helix upon binding of \(c\)Pml1p (Fig. 2F). The ensemble
of Snu17p conformations in this region is therefore ready to accept the incoming Pml1p. On the other hand, we did not detect a structural perturbation of hcBud13p in the dimeric complex with Snu17p when compared to the cRES trimer. Small 1H-15N HSQC chemical shift changes (Fig. 4A) were probably caused by a change in the environment associated with the lack of cPml1p and the unfolding of the C-terminal region of Snu17p (Fig. 4B,C).

Next, we analyzed the differences between the structure of the cPml1p–cSnu17p dimer and the cRES trimer (Fig. 4C). Comparison of the two structures revealed increased disorder of the C-terminal part of cPml1p associated with a loss of $\alpha$-helical character (Figs 2B,C and 4C,D). Moreover, the cPml1p position was slightly modified in response to cBud13p binding (Fig. 4C). The changes observed in the structure of the cPml1p–cSnu17p dimer were supported by 1H-15N HSQC spectra: chemical shifts of backbone amides at the C-terminal part of cPml1p differ depending on the presence of cBud13p (Fig. 4E) and reflect both changes in the level of disorder and structural changes.

Both cPml1p and hcBud13p represent largely the fragments that are necessary for binding to cSnu17p. However, the rest of each sequence could, in the context of the spliceosome, play a role in modulating the assembly of the RES complex. For example, the FHA domain of Pml1p is separated by only a short, six-residue linker from the Pml1p region, which binds to Snu17p. Notably, the phosphopeptide binding site of the FHA domain occurs in proximity to this linker. Alternatively, additional parts of Bud13p, which is intrinsically disordered along its complete sequence, might fold upon binding to other spliceosomal proteins in the context of the spliceosome. Currently, the order of RES complex assembly is not known.
Figure 5. Architecture of central tryptophan containing motifs (CTCMs) in RRM domains. (A) Comparison between the side chain position of a key tryptophan in different CTCMs bound to RRM domains. CTCMs are labelled 1, dark green, D-Acinus–RNPS1 (PDB code: 4A8X)31; 2, red, SF3b155(ULM5)–SPF45 (PDB code: 2PEH)29; 3, orange, SF1–U2AF65 (PDB code: 1OPI)30; 4, light green, EEL3J–EEL3B (PDB code: 2KR(B)32; 5, yellow, ICP27–REF2 (PDB code: 2KT5)33; 6, light blue, ORF57–REF2 (PDB code: 2YKA)34; 7, dark blue, hcBud13p–Snu17p dimer. Only α-helices 1 and 2 of the RRM are shown for clarity. (C) Schematic representation of the folding-upon-binding mechanism as seen in the assembly of the RES complex.
known, but given the two orders of magnitude higher affinity of Bud13p to Snu17p one can speculate that Bud13p might bind first, followed by Pml1p.

**Continuum of UHM-ULM-like interactions.** We then compared the non-canonical position of tryptophan 232 of Bud13p as observed in both the dimeric complex with Snu17p and the RES trimer (Fig. 5) and10,12, with other known RRM-peptide interactions. A canonical UHM–ULM interaction, in which a tryptophan residue is buried in a hydrophobic pocket of the RRM domain was observed for example for the complexes of splicing factor 3b (SF3b155(ULM5)) with alternative splicing factor 45 (SFPSF45) and of splicing factor 1 (SF1) with the large subunit of U2 snRNP auxiliary factor (U2AF6525,30). On the other hand, the complex structures of Acinus with RNA binding protein with serine rich domain 1 (RNPS131), eukaryotic translation initiation factors 3 J and 3b (EIF3–EIF3b25,32), infected cell protein 27 (ICP27) with RNA export factor2 (REF225) and immediate-early phosphoprotein from Saimiriine Herpes Virus ORF57 with REF225 do not have this canonical interaction. In these complexes—as well as in the Snu17p–Bud13p interaction—a conserved tryptophan residue is important for RRM binding, but its position is variable (Fig. 5A). In addition, the part of the protein, which is in contact with the RRM domain, samples a range of conformations (Fig. 5B). Most similar to the Bud13p–Snu17p recognition mode is the REF2–ICP27 complex, where the tryptophan side chain occupies a region near the C-terminus of α-helix 2 of the RRM domain (Fig. 5A). Intriguingly, ORF57, another REF2 binder, bares a degree of structural similarity to Bud13p as observed in the cPml1p–cSnu17p dimer structure. Notably, both Pml1p and REF2 are proteins involved in mRNA export33–35. The analysis suggests that there is a structural continuum of how tryptophan containing motifs bind to RRM domains.

In summary, we provided high-resolution structural evidence that the dimeric intermediates along the RES assembly pathway are not a simple structural equivalent of subtracting a given binding partner (Bud13p or Pml1p) of Snu17p from the RES trimeric complex. Instead, a number of localized structural changes are required for successive binding. The local structural changes are further accompanied by the large-scale rearrangement of the C-terminal part of Snu17p, which only holds into a stable α-helix upon interaction with Pml1p. The stepwise rigidification of the Snu17p structure upon binding of Bud13p and Pml1p provides a basis for the strong cooperative nature of RES assembly and RNA binding (Fig. 5C).

**References**

1. Will, C. L. & Luhrmann, R. Spliceosome structure and function. *Cold Spring Harb. Perspect. Biol.* 3, a003707 (2011).
2. Brow, D. A. Allosteric cascade of spliceosome activation. *Annu. Rev. Genet.* 36, 333–60 (2002).
3. Williams, S. G. & Hall, K. B. Linkage and Allostery in snRNP Protein/RNA Complexes. *Biochemistry* 53, 3529–39 (2014).
4. Williams, S. G. & Hall, K. B. Binding Affinity and Cooperativity Control U2B′/snRNA/U2A′ RNP Formation. *Biochemistry* 53, 3727–37 (2014).
5. Hennig, J. et al. Structural basis for the assembly of the Sxl-Unr translation regulatory complex. *Nature* 515, 287–290 (2014).
6. Dziembowski, A. et al. Proteomic analysis identifies a new complex required for nuclear pre-mRNA retention and splicing. *EMBO J.* 23, 4847–56 (2004).
7. Zhou, Y., Chen, C. & Johannsson, M. J. The pre-mRNA retention and splicing complex controls tRNA maturation by promoting TAN1 expression. *Nucleic Acids Res.* 41, 5669–78 (2013).
8. Tuo, S., Nakashima, K. & Pringle, J. R. Apparent defect in yeast bud-site selection due to a specific failure to splice the pre-mRNA of a regulator of cell-type-specific transcription. *Plos one* 7, e47621 (2012).
9. Scherrer, F. W., Jr & Spingola, M. A subset of Mer1p-dependent introns requires Bud13p for splicing activation and nuclear retention. *RNA* 12, 1361–72 (2006).
10. Tripsianes, K. et al. A Novel Protein-Protein Interaction in the RES (REtention and Splicing) Complex. *The Journal of biological chemistry* 289, 28640–50 (2014).
11. Trowitzsch, S., Weber, G., Luhrmann, R. & Wahl, M. C. An unusual RNA recognition motif acts as a scaffold for multiple proteins in the pre-mRNA retention and splicing complex. *J. Biol. Chem.* 283, 32317–27 (2008).
12. Wysoczanski, P. et al. Cooperative structure of the heterotrimeric pre-mRNA retention and splicing complex. *Nat. Struct. Mol. Biol.* 21, 911–8 (2014).
13. Wang, Q., Hu, J., Lynn, B. & Rymond, B. C. Interactions of the yeast SF3b splicing factor. *Mol. Cell. Biol.* 25, 10745–54 (2005).
14. Sattler, M., Schlescher, J. & Gregor, C. Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. *Prog. Nucl. Magn. Reson. Spectrosc.* 34, 93–158 (1999).
15. Bax, A., Clore, G. M. & Gronenborn, A. M. 1H-1H correlation via isotropic mixing of 13C magnetization, a new three-dimensional approach for assigning 1H and 13C spectra of 13C-enriched proteins. *J. Magn. Reson.* 88, 425–431 (1990).
16. Yamazaki, T., Forman-Kay, J. D. & Kay, L. E. Two-dimensional NMR experiments for correlating carbon-13/δ.ε. chemical shifts of aromatic residues in 13C-labeled proteins via scalar couplings. *J. Am. Chem. Soc.* 115, 11054–11055 (1993).
17. Zwahlen, C. et al. Methods for Measurement of Intermolecular NOEs by Multinuclear NMR Spectroscopy: Application to a Bacteriophage Φ N- Peptide/boxB RNA Complex. *J. Am. Chem. Soc.* 119, 6711–6721 (1997).
18. Delaglio, F. et al. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* 6, 277–93 (1995).
19. Vranken, W. F. et al. The CCPN data model for NMR spectroscopy: Development of a software pipeline. *Proteins* 59, 687–696 (2005).
20. Yao, L., Ying, J. & Bax, A. Improved accuracy of 15N-1H scalar and residual dipolar couplings from gradient-enhanced IPAP-HSQC experiments on protonated proteins. *J. Biomol. NMR* 43, 161–70 (2009).
21. Zweckstetter, M. NMR: prediction of molecular alignment from structure using the PALES software. *Nature protocols* 3, 679–90 (2008).
22. Guntert, P., Mumenthaler, C. & Wuthrich, K. Torsion angle dynamics for NMR structure calculation with the new program DYANA. *J. Mol. Biol.* 273, 283–298 (1997).
23. Schwiers, C. D., Kuskey, J. J., Tjandra, N. & Clore, G. M. The Xplor-NIH NMR molecular structure determination package. *J. Magn. Reson.* 160, 65–73 (2003).
24. Shen, Y. & Bax, A. Protein backbone and sidechain torsion angles predicted from NMR chemical shifts using artificial neural networks. J. Biomol. NMR 56, 227–41 (2013).
25. Doreleijers, J. F. et al. CING: an integrated residue-based structure validation program suite. J. Biomol. NMR 54, 267–283 (2012).
26. Baker, N. A., Sept, D., Joseph, S., Holst, M. J. & McCammon, J. A. Electrostatics of nanosystems: application to microtubules and the ribosome. Proc. Natl. Acad. Sci. USA 98, 10037–41 (2001).
27. Schwiers, C. D. & Clore, G. M. The VMD-XPLOR visualization package for NMR structure refinement. J. Magn. Reson. 149, 239–44 (2001).
28. Collinet, R. et al. Strategies for the structural analysis of multi-protein complexes: lessons from the 3D-Repertoire project. J. Struct. Biol. 175, 147–58 (2011).
29. Corsini, L. et al. U2AF-homology motif interactions are required for alternative splicing regulation by SPF45. Nat. Struct. Mol. Biol. 14, 620–9 (2007).
30. Selenko, P. et al. Structural basis for the molecular recognition between human splicing factors U2AF65 and SF1/mBBP. Mol. Cell 11, 965–76 (2003).
31. Murachelli, A. G., Ebert, I., Basquin, C., Le Hir, H. & Conti, E. The structure of the ASAP core complex reveals the existence of a Pinin-containing PSAP complex. Nat. Struct. Mol. Biol. 19, 378–86 (2012).
32. Elantak, L. et al. The indispensable N-terminal half of eIF3b/PR1-RRM and with eIF4A in stringent AUG selection. J. Mol. Biol. 396, 1097–116 (2010).
33. Tunnilcliffe, R. B. et al. Structural basis for the recognition of cellular mRNA export factor REF by herpes viral proteins HSV-1 ICP27 and HVS ORF57. PLoS Path. 7, e1001244 (2011).
34. Tunnilcliffe, R. B., Hautbergue, G. M., Wilson, S. A., Kalra, P. & Golovanov, A. P. Competitive and cooperative interactions mediate RNA transfer from herpesvirus saimiri ORF57 to the mammalian export adaptor ALYREF. PLoS Path. 10, e1003907 (2014).
35. Chekanova, J. A., Abruzzi, K. C., Rosbash, M. & Belostotsky, D. A. Sus1, Sac3, and Thp1 mediate post-transcriptional tethering of active genes to the nuclear rim as well as to non-nascent mRNP. RNA 14, 66–77 (2008).
36. Palancade, B. et al. Sumoylating and desumoylating enzymes at nuclear pores: underpinning their unexpected duties? Trends Cell Biol. 18, 174–83 (2008).

Acknowledgements
We thank K. Giller for the preparation of expression constructs. This work was supported by the German Science Foundation [Collaborative Research Center 860 project B2 to M.Z.].

Author Contributions
P.W. performed NMR measurements, data analysis and structure calculation. P.W. and M.Z. wrote the manuscript. S.B. and M.Z. supervised the study.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wysoczanski, P. et al. Structures of intermediates during RES complex assembly. Sci. Rep. 5, 12545; doi: 10.1038/srep12545 (2015).

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