Targeting Human Retinoblastoma Binding Protein 4 (RBBP4) and 7 (RBBP7)

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Abstract

RBBP4 and RBBP7 (RBBP4/7) are highly homologous nuclear WD40 motif containing proteins widely implicated in various cancers and are valuable drug targets. They interact with multiple proteins within diverse complexes such as NuRD and PRC2, as well as histone H3 and H4 through two distinct binding sites. FOG-1, PHF6 and histone H3 bind to the top of the donut shape seven-bladed β -propeller fold, while SUZ12, MTA1 and histone H4 bind to a pocket on the side of the WD40 repeats. Here, we briefly review these six interactions and present binding assays optimized for medium to high throughput screening. These assays enable screening of RBBP4/7 toward the discovery of novel cancer therapeutics.

Introduction

Retinoblastoma binding protein 4 (RBBP4) and 7 (RBBP7) also known as Retinoblastoma Associated protein 48 (RbAp48) and 46 (RbAp46), respectively, are highly homologous nuclear WD40 motif containing proteins^{1, 2}. RBBP4 and 7 (RBBP4/7) have been widely implicated in various cancers and are valuable drug targets. Elevated levels of RBBP7 in nonsmall cell lung cancer, and inhibition of migration ability of lung cancer cells upon RBBP7 knockdown have been reported³. RBBP7 is also overexpressed in other cancers such as renal cell carcinoma, medulloblastoma and breast cancer⁴⁻⁶. Upregulation of RBBP7 in bladder cancer results in a cell invasion phenotype, and a decrease in its expression inhibited cell invasion⁷. RBBP4 is also overexpressed in several cancers including hepatocellular carcinoma⁸, and acute myeloid leukemia^{9, 10}. Reduction of RBBP4 expression using siRNA resulted in the suppression of tumorigenicity. In human thyroid carcinoma, RBBP4 expression is upregulated and its

downregulation suppressed tumorigenicity¹¹. RBBP4 suppression in glioblastoma cells also enhanced sensitivity towards temozolomide chemotherapy¹².

RBBP4 and RBBP7 are highly homologous proteins with similar structures (89% sequence identity). They are donut shaped proteins with a seven-bladed β -propeller fold typical for WD40-repeat proteins. Distinct features include a prominent N-terminal α -helix, a negatively charged PP-loop and a short C-terminal α -helix. Two binding sites have been identified on these two proteins, a c-site on the top of the WD40 repeats (Fig.1A)¹³⁻¹⁵ and another located on the side of the WD40 domain between the N-terminal α -helix and PP-loop (Ser-347 to Glu-364 in RBBP7) (Fig. 1B)¹⁵⁻¹⁷. RBBP4/7 are components of several multi-protein complexes involved in chromatin remodeling, histone post-translational modifications and regulation of gene expression suggesting diverse functions for RBBP4/7. Such complexes include nucleosome remodeling and deacetylase (NuRD) complex¹⁸, switch independent 3A (Sin3A)¹⁹, and polycomb repressive complex 2 (PRC2)^{20, 21}. Some interaction partners of RBBP4/7 include Metastasis Associated Protein 1 (MTA1)^{18, 22}, also a component of NuRD complex, and suppressor of zeste 12 (SUZ12)^{15, 21} within the PRC2 complex. Structural studies have shown that these interactions along with binding to histone H4¹⁷ are through the side pocket on the WD40 domain of RBBP4/7 and Nurf55 (*Drosophila* RBBP4/7) (Fig. 1B). In addition, the C-terminal α -helix may be involved in binding of peptides to this site. This pocket is unique to RBBP4/7. It has both negatively charged and hydrophobic areas (Fig. 2). As a result, hydrophobic interactions play important roles in Su(z)12-Nurf55 binding. It has been shown that binding of H4, MTA1 and Su(z)12 to RBBP4/7 and Nurf55 are mutually exclusive^{15, 16}. Interestingly, RBBP4/7 are able to bind to MTA1, H3 or FOG-1 (Friend of GATA-1) independently. However, within the NuRD

complex, the presence of MTA1 can destabilize the interaction of RBBP4/7 with histones H3-H4¹⁶.

FOG-1, PHF6 (Plant homeodomain finger protein 6) and histone H3 bind to the top of the seven-bladed β -propeller fold. Crystal structures of RBBP4 in complex with PHF6¹³, FOG-1¹⁴, and Nurf55 with histone H3¹⁵ peptides reveal these ligands bind to the same position in a c-site on the top of the WD40 repeats, forming multiple hydrogen bonds. This site is negatively charged, creating a surface perfectly suited for interaction with positively charged residues of the peptides (Fig. 2). In all cases, one arginine of the peptide (Arg-2 in H3, Arg-163 in PHF6 and Arg-4 in FOG-1) is directly inserted into the negatively charged central channel of RBBP4/7 and forms a hydrogen bond with Glu-231 (Glu-235 in Nurf55) (PDB ID: 2YBA, 4R7A and 2XU7). Furthermore, all peptides adopt the same binding pose (Fig.1A). This observation suggests that PHF6, FOG-1 and H3 interactions with RBBP4/7 and Nurf55 are highly conserved.

Interacting proteins

MTA1

The NuRD complex represses the transcription of several genes. It consists of several proteins, HDAC1/2 (histone deacetylase), CHD3/4/5 (chromodomain-helicase-DNA-binding protein), MBD2/3 (methyl-CpG-binding domain protein), MTA1/2/3, RBBP4/7 and Gata2a/2b (GATA binding protein)^{23, 24}. MTA1, 2 and 3 do not co-exist in the NuRD complex but that complex composition could rather be cell-type specific²⁵⁻²⁸. MTA acts as a scaffolding protein and directly interacts with HDAC1/2 and RBBP4/7. The ELM2 domain of MTA1 mediates dimerization, and

its ELM2-SANT domain is responsible for recruiting HDAC1/2. The C-terminus region contains binding sites for RBBP4/7 and CHD4 (reviewed in ²⁹).¹⁸

MTA1 has been reported to be a highly dysregulated oncogene contributing to cancer progression and metastasis (reviewed in ³⁰). It is upregulated in various types of cancers, including breast, ovarian and colorectal cancer, hepatocellular carcinoma and B-cell lymphomas³¹. MTA1 is a member of the MTA family and similar to MTA2 and 3, it consists of several alternatively spliced forms (reviewed in ³²). So far, studies have largely been conducted with the full-length proteins and the roles of the spliced isoforms are yet to be explored in detail^{31, 33}. The N-terminus region of these proteins is well conserved, while the C-terminus differs more significantly. The conserved N-terminus consists of four domains, BAH (Bromo adjacent homology), ELM2 (EGL-27 and MTA1 homology), SANT (SWI, ADA2, N-CoR, TFIIB-B) and the GATA-like zinc finger domains. The variable C-terminus, consists of a Src-homology 3 binding domain, an acidic region, and a bipartite nuclear localization sequence (NLS; two in case of MTA1 and 2, one in case of MTA3) (reviewed in ³²). The expression of MTA1 in normal cells significantly varies from tissue to tissue, and is relatively higher in normal testis, brain, liver and kidney suggesting a role in these tissues (reviewed in ^{31, 34}). MTA1 is mainly nuclear, but studies have reported its expression both in the nucleus and in the cytoplasm of normal and cancerous cells. Apart from being diffused in the cytoplasm, it has been shown to be associated with microtubules and with the nuclear envelope in some cases (reviewed in ³¹).³⁵ MTA proteins have also been shown to interact with DNA and other co-regulators of the NuRD complex. Additionally, MTA1 serves NuRD-independent cellular functions. For example, it can associate with NURF (nucleosome remodeling factor) to activate gene expression. The

methylation status of MTA1 has been reported to be a key factor for determining complex association, as methylated MTA1 recruited NuRD, while demethylated MTA1 recruited NURF (reviewed in ³¹).

It has been shown that MTA1 has two regions with similar sequences that can independently bind RBBP4, and recruits two copies of RBBP4 in the NuRD complex^{18, 22}. Structural studies of complexes containing full-length RBBP4 and each of these two MTA1 regions show that the ‘side’ surface of the RBBP4 β -propeller is used for MTA1 interaction^{16, 18}. Testing MTA1 peptides with various lengths (656-686, 670-695 and 670-711) for binding to full length RBBP4 by isothermal calorimetry resulted in K_D values of 2.3 ± 0.3 , 0.05 ± 0.007 and 0.24 ± 0.16 μ M, respectively¹⁶. Values for MTA1 (656-686) are provided in Table 1 for comparison.

SUZ12

PRC2 (Polycomb Repressive Complex) catalyzes the trimethylation of histone H3 lysine 27 (H3K27), which results in transcriptional silencing^{36, 37}. PRC2 consists of a core complex of three subunits, EZH1/2 (enhancer of zeste homologue 1/2, the catalytic histone methyltransferase subunit), EED (embryonic ectoderm development) and SUZ12 (suppressor of zeste 12). Additional proteins interact with the core complex, including RBBP4/⁷³⁸. EZH2 activity requires interaction with both EED and SUZ12³⁹. Overexpression of EZH2 has been reported in prostate and breast cancer which is associated with advanced stage and poor prognosis. Similarly, EZH2 is upregulated in various solid malignancies including renal cell carcinoma⁴⁰, lung⁴¹, hepatocellular⁴² and pancreatic⁴³ cancers. Knock-out studies performed with respect to the components of the PRC2 complex in mice and flies show that PRC2 and all its components are

essential for viability as the knockouts exhibit embryonic lethality⁴⁴⁻⁴⁶. Thus, PRC2 is essential during the early stages of embryogenesis and it regulates the expression of several developmental genes^{47, 48}. A study from Suz12 conditional knockout mice shows that Suz12 is important for the maintenance of hematopoietic stem cells and required for the development of the lymphoid lineage but not myeloid cells⁴⁹. AEBP2 which serves as an associating component of PRC2 complex also interacts with RBBP4 and SUZ12, stabilizing the core PRC2 complex and increasing the methyltransferase activity of PRC2.³⁹ It has been shown that the three zinc fingers and RRK rich motif of AEBP2 interact with RBBP4.⁵⁰ RBBP4 binds to AEBP2 (379–390) peptide with a dissociation constant of $7.6 \pm 0.6 \mu\text{M}$ through the c-site on the top of the WD40 repeats.⁵⁰ The crystal structures of heterotetrameric complex consisting of SUZ12, RBBP4, Jarid2, and AEBP2 fragments have recently been reported as well.⁵¹

BCL11A is another protein that interacts with PRC2 and NuRD and SIN3A complexes through binding to RBBP4. BCL11A competes with histone H3 for binding to the top binding site on RBBP4. However, unlike histone H3, the crystal structure of RBBP4 in complex with a BCL11A peptide (residues 2–16) shows additional novel interactions between BCL11A and the side of the propeller of RBBP4 through residues 14 to 16 of BCL11A.⁵²

PHF6

PHF6 is an X-chromosome encoded protein with nuclear and nucleolar localization, which is highly conserved among vertebrates. It consists of two atypical zinc-finger domains, called extended PHD (ePHD) domains (zinc knuckle, C2HC-type coordinating one zinc ion and an atypical PHD or ZaP)⁵³⁻⁵⁵. ePHD1 and ePHD2 share a sequence identity of 49%⁵⁶. Although the canonical PHD domain can bind to histones, studies with ePHD2 have shown that it does not

bind to any histone, but can bind to dsDNA in a non-sequence-specific manner⁵⁴. From murine studies, PHF6 has been shown to be highly expressed in the embryonic central nervous system including brain and anterior pituitary, and its localization shifting from nucleus and cytoplasm during development to exclusively nuclear in adult⁵⁷. Knockdown studies indicate a role for PHF6 in cell proliferation and neuronal migration^{58, 59}. Clinically, *PHF6* mutations have been implicated in two intellectual disability disorders, namely, Börjeson–Forssman–Lehmann Syndrome (BFLS) and Coffin–Siris Syndrome, in addition to T-Cell acute lymphoblastic leukemia (T-ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), hepatocellular carcinoma, B-cell acute lymphoblastic leukemia (B-ALL), myelodysplastic syndromes and myeloid neoplasmas.⁶⁰⁻⁷⁰ PHF6 is a potential oncogene which is overexpressed in various cancers such as colon, breast, stomach and glioma⁷¹. Its suppression in B-cell malignancies has been reported to impair tumor progression⁷². Lower expression of PHF6 has also been reported in some other cancers such as esophagus cancer⁷¹.

PHF6 interaction partners (NuRD complex, PAF-1 and UBF) exist both in the nucleoplasm and the nucleolus. From the NuRD complex, PHF6 was shown to co-purify with CHD3, CHD4, RBBP4, RBBP7 and HDAC1, wherein, PHF6 interaction with RBBP4 and CHD4 was restricted to the nucleoplasm, despite its presence in the nucleolus as well⁵³. Among the various co-purified components of the NuRD complex, a direct interaction has been reported between RBBP4 and PHF6 and the interaction has been characterized both structurally and biophysically^{13, 54}. A structure of full-length RBBP4 in complex with a PHF6 peptide (157-171 amino acids) showed that a negatively charged surface at the top of the RBBP4 β -propeller is essential for binding to a PHF6 peptide (a dissociation constant of $7.1 \pm 0.4 \mu\text{M}$; Table 1)¹³. Functionally, it was shown

that the PHF6 region spanning amino acids 145 to 207 was sufficient for repressing the genes to which it was targeted, and its interaction with RBBP4 was required for the process^{13, 54}.

FOG-1

FOG-1 is a 1006 amino acid protein that consists of nine zinc fingers, which are involved in mediating interactions with other proteins⁷³⁻⁷⁶. Within the NuRD complex, FOG-1 has been shown to directly interact with RBBP4. This interaction has been characterized biophysically and structurally. The N-terminus residues of FOG-1 (1-12 amino acids) have been shown to bind to RBBP4 with a K_D of $0.6 \pm 0.13 \mu M$ ¹⁴ (Table 1).

FOG-1 has been shown to be largely functional in the hematopoietic system, in addition to other tissues such as male gonads, heart, and intestine (reviewed in⁷⁷). Accordingly, it is important for erythropoiesis and megakaryopoiesis and repressing myelo-lymphoid differentiation⁷⁸. FOG-1 knockout mice are embryonically lethal due to severe anemia⁷⁹. FOG-1 interaction with GATA1 has been well studied and reports also suggest its co-expression with GATA2, 3 and 4 (reviewed in⁷⁷). Disruption in its interaction with GATA-1 results in familial dyserythropoietic anemia and thrombocytopenia⁸⁰. FOG-1 has been shown to act both as a co-activator or a co-repressor for its target genes (summarized in⁷⁷).⁸¹ FOG-1 is also capable of upregulating the expression of genes that are otherwise repressed by GATA3⁸². In addition to GATA factors, FOG-1 interacts with, and recruits co-repressor complexes, NuRD complex and C-terminal binding protein (CtBP)^{83, 84}.

Binding to Histone H3 and H4

Structural data shows that the ‘side’ surface of RBBP7 (and Nurf55) β -propeller interacts with a H4 peptide and this interaction requires unfolding of helix 1 of histone H4. It has been suggested

that this unfolding would be required when H3 is present alone or in a complex (for example, in the nucleosome, or with H3 and ASF-1)^{17, 85, 86}. On the other hand, an H3 peptide interacts with the ‘top’ surface of the Nurf55 β -propeller and thus, H3 and H4 peptides bind at different surfaces^{15, 86}. Interactions between Nurf55/RBBP4/RBBP7 and H3 and H4 in the H3-H4 dimeric and tetrameric complexes have been extensively characterized^{17, 85-87}. Murzina and colleagues demonstrated that the K_D values for RBBP7 interaction with recombinant full-length H3-H4 complex and H4 peptide (16-41 amino acids) were 0.9 and 1.1 μ M, respectively, as observed by fluorescence spectroscopy¹⁷. However, the N-terminal tail of H4 (1-15 amino acids) is not involved in these interactions⁸⁶. Nurf55, on the other hand, interacts with H3 N-terminal tail peptide (1-15 amino acids) with a K_D of 0.8 ± 0.1 μ M as measured by fluorescence polarization (FP)¹⁵. It has also been shown that modifications of the H3 peptide (1-15 amino acids), such as H3K4me3 and H3K9me3, reduced this interaction⁸⁶. A similar observation was made for the interaction of RBBP4 with acetylated H4 peptide⁸⁷. Apart from the N-terminal tails, H3 and H4 can interact with RBBP4 *via* other surfaces, though these interactions are weaker⁸⁷. A recent study using RBBP4 has reported that the protein interacts with a H3-H4 dimer but not the tetramer, and the K_D for the interaction was 0.61 ± 0.49 nM⁸⁷. Though RBBP4 and RBBP7 interact with histone proteins directly (H3 and H4), this interaction is affected by other RBBP4/7 binding partners. Interaction with NuRD is believed to recruit this complex to nucleosomes.

Developing a suite of assays for high throughput screening

As discussed, the dysregulation of RBBP4/7 have been widely implicated in cancers. However, no small molecule antagonist of their interactions with any of the interacting proteins has been reported to-date. This necessitates developing binding assays and optimizing them for high

throughput screening toward the discovery of potent, selective and cell permeable small molecules to further investigate the roles they play in normal cells and in cancers. We aimed at the development of fluorescence polarization-based RBBP4/7 binding assays with the 6 peptides which have already been characterized, and crystallized in complex with RBBP4 or RBBP7; MTA1 (656-686; DVFYIMATEETRKIRKLLSSSETKRAARRPYK)¹⁶, SUZ12 (105-121; LIAPIFLHRTLTYMSHR)²¹, H3 (1-21; ARTKQTARKSTGGKAPRKQLA; binding of peptides with different length was reported by Schmitges et. al.¹⁵), FOG-1 (1-15; MSRRKQSNPRQIKRS)¹⁴, PHF6 (157-171; KSKKKSARKGRPRKTN)¹³ and H4 (16-41; KRHRKVLRDNIQGITKPAIRRLARRG)¹⁷ (Fig. 1A and 1B). Available binding data for these peptides are summarized in Table 1. They bind to RBBP4/7 with a range of affinities from a K_D value of 35 nM for Nurf55 tight binding to H4 (26-45) peptide to low micromolar K_D for SUZ12 binding to histone H3. We attempted to develop binding assays for all these interactions and optimize them for screening in a 384-well format. Binding of each FITC-labeled peptide to RBBP4/7 was tested at various pH (Supplementary Figure 1A-6A) and buffers (Supplementary Figure 1B-6B). Binding of MTA1, SUZ12, histone H3 and H4 peptides generated enough signal to allow the development of assays close to physiological conditions (pH 7 to 8). The assays were performed using selected buffer and pH at various buffer concentrations (Supplementary Figure 1C-6C). Interestingly, the presence of up to 100 mM of salt (NaCl/KCl) did not have any effect on FP signal for peptides that bind to the side pocket (Supplementary Figure 1D-3D). However, for peptides that bind to the c-site on top of the WD40 fold, the presence of salt as high as 25 mM significantly decreased the binding (Supplementary Figure 4D-6D). These effects are consistent with the highly negatively charged c-site and the highly hydrophobic side pocket (Fig. 2). We further evaluated the effect of detergents (Triton X-100, Tween 20 and NP-40) as

well as reducing agents (DTT and TCEP) (Supplementary Figure 1E and 1F through 6E and 6F). Optimum buffer conditions selected for performing each assay are shown in Table 2. Total fluorescence was also measured at various concentrations of FITC-labeled peptides to be able to select the lowest peptide concentration that generates enough signal (Supplementary Figure 1G to 6G). This is particularly important for tight interactions. The presence of up to 5% DMSO in assay buffers did not have a dramatic effect on FP signal and was well tolerated with all of the peptides (Supplementary Figure 1H to 6H).

Using the optimized assay conditions we assessed binding and determined the apparent K_D values for the interaction of MTA1 (33 ± 13 nM), SUZ12 (6.4 ± 0.6 μ M), PHF6 (201 ± 102 nM), FOG1 (488 ± 245 nM) and histone H3 (1.4 ± 0.2 μ M) peptides to RBBP4 (Fig. 3). Binding of histone H4 peptide (16-41) to RBBP7 was also tested with a K_D value of 135 ± 12 nM (Fig. 3C). Typically, FP-based screening relies on displacement of peptide from the binding site on the target protein by small molecules that compete with peptide^{88, 89}. To discriminate against the term IC_{50} value, which indicates the concentration of the compound that causes 50% reduction in activity of the target enzyme, we have previously introduced the term “ K_{disp} ” which refers to the same concept but for displacement-based binding assays⁸⁸⁻⁹⁰. To evaluate displacement of the peptides from RBBP4/7 binding sites, we used unlabeled version of each peptide to compete and reduce the signal (Fig. 4). Clear displacement of FITC- labelled peptides was observed for MTA1 (K_{disp} : 308 ± 15 nM), histone H4 (K_{disp} : 662 ± 8 nM), PHF6 (K_{disp} : 1.7 ± 0.2 μ M), and FOG1 (K_{disp} : 6.8 ± 3.3 μ M) peptides (Fig. 4A, 4C, 4D and 4E). All these peptides bind with K_D values below 500 nM (Fig. 3). For SUZ12 binding to RBBP4 with K_D values of 6.4 μ M, we detected no displacement by unlabeled peptide up to 10 μ M (Fig. 4B). This is probably because the range of concentrations used may not be high enough. However, we were unable to use

higher concentrations of SUZ12 peptide due to low solubility. For histone H3 peptide, we only included a range of up to 1.5 μ M (Fig. 4F) to test the displacement as the signal at higher concentrations of peptide was dramatically fluctuating.

Although we could not show that the displacement of SUZ12 and histone H3 peptides is possible, we did not dismiss the assays for screening. Performing Z' -determination for assays with all six peptides (Fig. 5) indicated that the assay for RBBP7 with histone H4 has the highest Z' -factor (0.76; Fig. 5C) followed by RBBP4 with SUZ12 (0.63; Fig. 5B), histone H3 (0.59; Fig. 5F) and MTA1 (0.55; Fig. 5A). As expected, the assays for PHF6 and FOG-1 were not suitable for screening with Z' -Factors below 0.5 (Fig. 5D and 5E).

In conclusion, we provide assays for displacement of MTA1, SUZ12, histone H3 and H4 peptides that have been optimized for high throughput screening of RBBP4/7, enabling discovery of small molecule antagonists that could be used toward further investigating RBBP4/7 roles in cells and discovery of cancer therapeutics.

Contribution of authors

M.A designed and performed all experiments, analyzed data and wrote the manuscript. V.T performed structural analysis (figure 1 and 2). E.G purified proteins. M.V led the project, designed experiments, reviewed data and wrote the manuscript.

Acknowledgement

We would like to thank Drs. Matthieu Schapira and Peter Brown for critical review of the manuscript. The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, BoehringerIngelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA) [ULTRA-DD grant no. 115766], Janssen, Merck & Co., Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and the Wellcome Trust.

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Tables

Table 1. Interaction of RBBP4/7 with reported side-surface and top-surface binding peptides.

Protein	peptide	Assay	K _D (μM)	Structure availability	Binding site	References
RBBP4	MTA1 (656-686)	ITC	2.3±0.3	Yes	Side	Alqarni et. al., 2014 ¹⁶
Nurf55	Su(z)12 (75-93)	ITC	6.7 ± 0.3	Yes	Side	Schmitges et.al., 2011 ¹⁵
RBBP7	H4 (16-41)	Flu	1.1	Yes	Side	Murzina et.al., 2008 ¹⁷
Nurf55	H4 (26-45)	ITC	0.035 ± 0.001	Yes	Side	Nowak et. al., 2011 ⁸⁶
Nurf55	H3 (1-15)	FP	0.8 ± 0.1	Yes	Top	Schmitges et.al., 2011 ¹⁵
Nurf55	H3 (1-15)	ITC	2.1 ± 0.05	Yes	Top	Nowak et. al., 2011 ⁸⁶
Nurf55	H3 (1-28)	ITC	1.6 ± 0.1	Yes	Top	Nowak et. al., 2011 ⁸⁶
RBBP4	PHF6 (157-171)	ITC	7.1±0.4	Yes	Top	Liu et.al., 2015 ¹³
RBBP4	FOG-1 (1-12)	ITC	0.6 ± 0.13	Yes	Top	Lejon et.al., 2011 ¹⁴

Flu: fluorescence spectroscopy, FP: Fluorescence Polarization assay, ITC: Isothermal Titration Calorimetry.

Table 2. Optimized buffer conditions for the fluorescence polarization based assays for quantifying interaction of RBBP4/7 with their interacting peptides.

Interaction	Buffer	Protein (μM)	Peptide (nM)
RBBP4-MTA1 (656-686)	50 mM Sodium phosphate, pH 7.5, 150 mM KCl, 0.01 % NP-40	0.15	10
RBBP4-SUZ12 (105-121)	50 mM Potassium phosphate, pH 7.0, 75 mM KCl, 0.005% NP-40	12	20
RBBP4-H3 (1-21)	10 mM Tris, pH 8.0, 0.005 % NP-40	3	5
RBBP4-PHF6 (157-171)	25 mM Tris, H 7.5, 0.02 % TX-100, 2 mM DTT	0.30	5
RBBP4-FOG-1 (1-15)	50 mM Tris, pH 7.5, 0.005 % NP-40, 2 mM DTT	1.20	5
RBBP7-H4 (16-41)	25 mM Sodium phosphate, pH 8.0, 75 mM NaCl, 0.02 % TX-100	0.30	5

Figure legends

Figure 1. Structural details of RBBP4/7 interactions. (A) Structural alignment of RBBP4 in complex with PHF6 and FOG-1 (PDB code: 4R7A and 2XU7, respectively), and Nurf55 in complex with histone H3 peptide (PDB code: 2YBA). (B) Structural alignment of RBBP4 in complex with MTA1 (PDB code: 4PBY), RBBP7 in complex with histone H4 (PDB code: 3CFV), and Nurf55 in complex with Su(z)12 (PDB code: 2YB8). All crystal structures were downloaded from RCSB Protein Data Bank and analyzed with Accelrys Discovery studio 4.1 visualizer.

Figure 2. Two binding pockets on RBBP4/7. The negative charges and hydrophobic surface in the protein-binding pockets of the RBBP4/7. C-site on the *left* and side binding pocket on the *right*.

Figure 3. Assessing the interaction of human RBBP4/7 with peptides. Interaction of RBBP4 with FITC labelled (A) MTA1 (656-686), (B) SUZ12 (105-121), (D) PHF6 (157-171), (E) FOG-1 (1-15) and (F) histone H3 (1-21) was assessed by monitoring the increase in fluorescence polarization signal. The interaction of (C) RBBP7 with histone H4 (16-41) was assessed as well.

Figure 4. Displacement of interacting peptides. Displacement of all binding peptides shown in figure 1 using respective unlabeled peptides was assessed. FITC-labeled (A) MTA1, (D) PHF6 and (E) FOG-1 peptides were competed off RBBP4 by respective unlabeled peptides. Similarly (C) histone H4 peptide was displaced from RBBP7 binding site. Unlabeled peptide within the indicated ranges did not displace the FITC-labeled (B) SUZ12 or (F) histone H3 peptides from RBBP4 binding sites.

Figure 5. Z'-factor determination. Suitability of the peptide displacement assays for screening was determined by calculating the Z'-factor for interaction of RBBP4 with (A) MTA1 (0.55), (B) SUZ12 (0.63), (D) PHF6 (below 0.5), (E) FOG-1 (below 0.5) and (F) H3 (1-21) (0.59). (C) Screening RBBP7 using histone H4 resulted in a Z'-factor of 0.76.

Figure 1A

Figure 1B

Figure 2

Figure 3

Figure 4

Figure 5