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The N-terminal acetyltransferase Naa10/ARD1 does not acetylate lysine residues

Robert S. Magin,[1,2,3] Zachary M. March[1,3], and Ronen Marmorstein[1,2,4]

[1]Department of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA [2]Abramson Family Cancer Research Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, [3]Graduate Group in Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA [4]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA *Correspondence: marmor@mail.med.upenn.edu

Running Title: Naa10/Ard1 does not acetylate lysine residues

To whom correspondence may be addressed: Dr. Ronen Marmorstein, Department of Biochemistry & Biophysics, Abramson Family Cancer Research Institute, Perelman School of Medicine at the University of Pennsylvania, 421 Curie Blvd., Philadelphia, PA 19104, USA, Tel.: (215) 898-7740; Fax: (215) 746- 5511; email: marmor@mail.med.upenn.edu

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ABSTRACT

The N-terminal acetyltransferase NatA is a heterodimeric complex consisting of a catalytic subunit (Naa10/ARD1) and an auxiliary subunit (Naa15). NatA co-translationally acetylates the N-termini of a wide variety of nascent polypeptides. In addition, Naa10 can act independently to post-translationally acetylate a distinct set of substrates, notably actin. Recent structural studies of Naa10 also reveal the molecular basis for N-terminal acetylation specificity. Surprisingly, recent reports claim that Naa10 may also acetylate lysine residues of diverse targets, including methionine sulfoxide reductase A (MSRA), myosin light chain kinase (MLCK), and Runt-related transcription factor 2 (Runx2). Here, we used recombinant proteins to reconstitute and assess lysine acetylation events catalyzed by Naa10 *in vitro*. We show that there is no difference in lysine acetylation of substrate proteins with or without Naa10, suggesting that the substrates may be chemically, rather than enzymatically, acetylated. Together, our data argue against a role for Naa10 in lysine acetylation.

INTRODUCTION

Acetylation is a common and important protein modification in biology. It has roles in diverse biological processes including gene expression, metabolism, signal transduction and protein degradation (1). In general, there are two forms of protein acetylation; lysine acetylation of the epsilon amino group, and N-terminal acetylation, which occurs on the alpha amino group on the N-termini of proteins. These two types of acetylation are catalyzed by two different groups of enzymes: the lysine acetyltransferases (KATs) and the N-terminal acetyltransferases (NATs) (2). While lysine acetylation occurs on thousands of proteins to mediate diverse biological processes (3), N-terminal acetylation has also more recently been shown to be a ubiquitous and important modification, with a variety of functional consequences for a diverse array of cellular processes, such as protein localization (4), mediating protein-protein interactions (5), and regulating protein degradation (6).

There are five conserved NATs found in eukaryotes termed NatA-NatE. These enzymes differ in their N-terminal substrate profiles (2). NatA, which is responsible for the plurality of N-terminal acetylation (2), is a heterodimeric complex consisting of a catalytic subunit termed Naa10 (previously known as Ard1) and an auxiliary subunit termed Naa15 (previously known as NATH or Nat1) (7). NatA acetylates N-termini containing an N-terminal residue that has a small radius of gyration: alanine, cysteine, glycine,

serine, threonine, and valine (8). Structural and enzymatic studies reveal that the enzymatic subunit Naa10 has a different substrate profile when it is not in complex with the auxiliary subunit Naa15. As a monomer, Naa10 acetylates N-termini in which the N-terminal residues are acidic, such as actin, whose first three N-terminal residues are glutamates (9,10).

Recent reports claim to have identified lysine substrates for monomeric Naa10. These include the transcription factor Runx2 (11), the enzyme methionine sulfoxide reductase A (MSRA) (12), and myosin light chain kinase (MLCK) (13). These findings were surprising to us since the structure of all NATs determined to date (9,14-17), including Naa10 (9), contain an extended loop, which seem to occlude lysine side chains within a polypeptide from lying across the active site as they do in KATs (15-18). In addition, other reports demonstrating that Naa10 acetylates a lysine residue on Hif-1α have failed to be replicated (19-21), making the question of whether Naa10 is able to acetylate lysine residues controversial. We therefore performed a number of experiments to investigate this question. We found that the addition of acetyl-CoA did indeed promote the acetylation of MSRA and Runx2, but that this acetylation was Naa10-independent. Based on this observation, we conclude that lysine residues on these substrates are not acetylated by Naa10 but are instead acetylated by chemical means.

MATERIALS AND METHODS

Cloning and purification of Naa10—The gene encoding full length human Naa10 (residues 1-235) was cloned into a pETDUET vector containing an N-terminal His_6-SUMO tag. The resulting construct was transformed into Rosetta (DE3)pLysS competent *E. coli*. Cells were grown to OD_{600} 0.7-0.9 prior to inducing protein expression with 0.5 mM isopropyl β-d-1-thiogalactopyranoside (IPTG) at 16°C for ~16 hr. All subsequent purification steps were carried out at 4°C. Cells were isolated by centrifugation and lysed by sonication in lysis buffer containing 25 mM Tris, pH 8.0, 1 M NaCl, 10 mM β-mercaptoethanol (βME) and 10 μg/ml phenylmethanesulfonylfluoride (PMSF). The lysate was clarified by centrifugation and passed over 6 ml of Ni-NTA resin (Thermo Scientific), which was subsequently washed with 500 ml of lysis buffer supplemented with 25 mM imidazole. The protein was eluted in 50 ml of lysis buffer supplemented with 300 mM imidazole and dialyzed into ion exchange buffer containing 25 mM Tris pH 8.5, 50 mM NaCl, and 10 mM βME and loaded onto a 5 ml Q ion exchange column (GE Healthcare). The protein was eluted in the same buffer with a salt gradient (50-750 mM NaCl) over the course of 20 column volumes. Peak fractions were pooled, concentrated to 500 μl, and loaded onto an s200 gel filtration column (GE Healthcare) in sizing buffer containing 25 mM HEPES pH 7.0, 200 mM NaCl, and 1 mM DTT. Peak fractions were pooled and concentrated. Proteins were aliquotted, snap-frozen in liquid nitrogen, and stored at -80 °C for further use. A portion of the protein was then incubated with Ulp1 protease overnight to cleave off the His_6-SUMO tag in sizing buffer. After cleavage, this solution was subjected to an additional Ni-NTA purification step to remove Ulp1, His_6-SUMO, and any uncut Naa10 fusion protein. The resin was then washed with approximately ten column volumes of dialysis buffer supplemented with 25 mM imidazole, which was pooled with the initial flow-through. The protein was concentrated to 500 μl and run on a Superdex 75 gel filtration column. Peak fractions were pooled and concentrated. Proteins were aliquotted, supplemented with 5% glycerol, snap-frozen in liquid nitrogen, and stored at -80 °C for further use.

Cloning and purification of NatA and GST-Naa50—Human Naa10 (residues 1-160) and Naa15 (residues 1-866) constructs were engineered using a pFastBac dual vector. The Naa15 subunit contained an N-terminal His_6-tag. A bacmid was generated by transposition into DH10 bac competent *Escherichia coli* cells using the bac-to-bac system (Invitrogen). *Spodoptera frugiperda* (Sf9) cells cultured in SFM II medium were transfected with the Naa10/Naa15 bacmid using cellfectin reagent (Invitrogen). The resulting baculovirus was amplified until reaching a high titer. Sf9 cells were grown to a density of $1x10^6$ cells/ml and infected using the amplified Naa10/Naa15 baculovirus to an MOI (multiplicity of infection) of ~1-2. The cells were grown at 27°C and harvested 48 hours post-infection. All subsequent purification steps were carried out at

4°C. Cells were isolated by centrifugation and lysed by sonication in lysis buffer containing 25 mM Tris, pH 8.0, 1 M NaCl, 10 mM βME, 10 µg/ml PMSF, and DNase. The lysate was clarified by centrifugation and passed over 6 ml of Ni-NTA resin (Thermo Scientific), which was subsequently washed with 500 ml of lysis buffer supplemented with 25 mM imidazole. The protein was eluted in 50 ml of lysis buffer supplemented with 300 mM imidazole. The protein was concentrated to 500 µl, and loaded onto a Superdex 200 gel filtration column (GE Healthcare) in sizing buffer containing 25 mM HEPES pH 7.0, 200 mM NaCl, and 1 mM DTT. Peak fractions were pooled and concentrated. Proteins were aliquotted, snap-frozen in liquid nitrogen, and stored at -80 °C for further use. Human GST-Naa50 was purified as described (15) with the following changes: the protein was not cleaved with TEV protease and was run on a Superdex200 gel filtration column.

Cloning and purification of Runx2 and MSRA—The genes encoding the runt domain of mouse Runx2 (residues 186-315), and full length human MSRA (residues 1-235) were ordered from Bio Basic. These were both cloned into a modified pETDUET vector containing a TEV-cleavable, N-terminal GST tag. The resulting constructs were transformed into Rosetta (DE3)pLysS competent *E. coli*. Cells were grown to OD_{600} 0.7-0.9 prior to inducing protein expression with 0.5 mM IPTG at 16°C for ~16 hr. All subsequent purification steps were carried out at 4°C, and were identical for both proteins except where noted. Cells were isolated by centrifugation and lysed by sonication in lysis buffer containing 25 mM Tris, pH 8.0, 1 M NaCl, 10 mM βME and 10 µg/ml PMSF. The lysate was clarified by centrifugation and incubated with 6 ml glutathione resin (Thermo Scientific) for one hour. The resin was subsequently washed with 500 ml of lysis buffer. The protein was eluted in 50 ml lysis buffer supplemented with 20 mM reduced glutathione (Sigma) and dialyzed into ion exchange buffer containing 25 mM sodium citrate pH 5.5, 25 mM NaCl, and 10 mM βME and loaded onto a 5 ml SP ion exchange column (GE Healthcare). The protein was eluted in the same buffer with a salt gradient (25-750 mM NaCl) over the course of 20 column volumes. Peak fractions were pooled, concentrated to 500 µl, and loaded onto an s200 gel filtration column (GE Healthcare) in sizing buffer containing 25 mM HEPES pH 7.0, 200 mM NaCl, and 1 mM DTT. Peak fractions were pooled and concentrated. Half of the protein was then incubated on glutathione resin with TEV protease overnight in sizing buffer to cleave off the GST tag. After cleavage, the resin was washed with approximately ten column volumes of sizing buffer, and the untagged protein was collected. The protein was concentrated to 500 µl and run on a Superdex200 gel filtration column. Peak fractions were pooled and concentrated. Proteins were aliquotted, supplemented with 5% glycerol, snap-frozen in liquid nitrogen, and stored at -80 °C for further use.

Acetyltransferase assays against peptides—Acetyltransferase assays were carried out in sizing buffer. All peptides were ordered from GenScript. The peptides for the reported lysine substrates corresponded to ~20 residues surrounding putative acetylated lysine. These peptides were ordered with acetylated N-termini to ensure that signal would not result from N-terminal acetyltransferase activity. Tryptophan was added to the C-terminus to allow for accurate concentration determination. The peptides were as follows: MLCK- TVHEKKSSRKSEYLLPVAW, MSRA- RKEQTPVAAKHHVNGNRTVW, Runx2- QVATYHRAIKVTVDGPRW. The actin substrate peptide used was previously described in (9). In the assays, 300 µM of radiolabeled [^{14}C]acetyl-CoA (4 mCi/mmol; PerkinElmer Life Sciences), 1 µM Naa10 or NatA, and 1 mM peptide were reacted in 50 µl of total reaction volume for 4 hours at 37 °C. To quench the reaction, 15 µl of the reaction mixture was added to negatively charged P81 paper (Millipore), and the paper disks were immediately placed in wash buffer (10 mM HEPES, pH 7.5). The papers were washed three times, at 5 minutes per wash, to remove unreacted acetyl-CoA. The papers were then dried with acetone and added to 4 ml of scintillation fluid, and the signal was measured with a Packard Tri-Carb 1500 liquid scintillation analyzer.

Acetyltransferase assays against protein domains—Reactions were performed as above but with 50 µM substrate (Actin peptide, recombinant Runx2, or recombinant MSRA). 15 µl of 20% trichloroacetic acid (TCA) was added to 15 µl reaction mixture to precipitate acetylated substrates. The substrates were allowed to

precipitate on ice for 10 minutes. The precipitated substrates were pelleted by centrifuging for 5 minutes at 13,000 rpm. The supernatant was discarded and 200 µl of cold acetone was added to wash the pellets. These were incubated on ice for 5 minutes before centrifuging at 13,000 rpm. This wash step was repeated twice more. The pellets were added to 4 ml scintillation fluid, and the signal was measured with a Packard Tri-Carb 1500 liquid scintillation analyzer.

Acetyltransferase assays with increasing concentrations of acetyl-CoA—The assays were done as the TCA precipitation assays with the following changes. 1 mM of substrate (Actin peptide, recombinant Runx2, or recombinant MSRA) was used, and the amount of acetyl-CoA was varied (150 µM- 1000 µM). The reactions took place for 20 minutes at 37°C.

Protein pull downs—All pull downs were performed in sizing buffer. 1 µM of GST, GST-Runx2, GST-MSRA, or GST-Naa50 (positive control for NatA) were incubated with either 5 µM Naa10 or 5 µM NatA and 75 µl of glutathione resin in a total volume of 400 µl for 1 hour at 4 °C. The resin was washed with 5 ml of sizing buffer and eluted with 100 µl of buffer supplemented with 20 mM glutathione.

Acetyltransferase assay followed by Western blot—300 µM of acetyl-CoA (Sigma), 1 µM enzyme (Naa10 or hMof), 50 µM substrate (Runx2, MSRA, or the histone dimer H3/H4) were reacted in 20 µl of reaction volume for 4 hours at 37 °C. The reaction was performed in a buffer containing 50 mM Tris pH 8.0, 0.1 mM EDTA, 1 mM DTT, 10 mM sodium butyrate, 100 mM NaCl, and 10% glycerol. To quench the reaction, SDS loading dye was added. Reactions were diluted to 200 µl, and 20 µl of the dilution were run on the gel. Two different anti-acetyl-lysine antibodies were used (Cell Signaling (#9681) and ImmuneChem (ICP0380)). hMof and histones were prepared as described (22,23).

Results

The structure of Naa10 and other NATs are incompatible with lysine acetylation—A superposition of Naa10 (9) and the Gcn5 KAT (24) bound to their cognate N-terminal and lysine peptide substrates, respectively, illustrates why these enzymes are tailored to acetylate their respective substrate (**Figure 1A**). Specifically, the Gcn5 KAT bound to a histone tail peptide centered on the lysine 14 target reveals that the peptide sits across a wide surface groove of the enzyme with the central lysine 14 residue inserting into an a narrow active site cavity (24) (**Figure 1A**). The Hat1 histone acetyltransferase KAT bound to a histone H4 peptide shows a similar substrate-binding configuration (25). The Naa10 NAT and Gcn5 KAT superimpose fairly well (RMSD = 6.4) except for the substrate binding sites (RMSD = 10.17). Most notably, Naa10 contains an extended loop between β-strands β6 and β7, that is not present in Gcn5, that blocks peptide binding across the surface groove that is present in Gcn5 (**Figure 1A**). Instead, Naa10 contains an active site cavity that can only accommodate an N-terminal peptide (**Figure 1A**). A superposition of all available NAT structures (9,14-17) reveal that they contain substrate binding sites that are tailored for N-terminal substrate binding oriented similar to Naa10 and incompatible with binding lysine-containing substrates oriented similar to Gcn5 (**Figure 1B**).

Naa10 does not acetylate lysine residues within peptides from MLCK, MSRA, or RUNX2—Although the structural superpositions described above argue against Naa10 acetylation of lysine substrates, it remains possible that Naa10 may be able to adopt an alternative conformation to accommodate lysine substrate acetylation. To address this possibility, we first tested whether recombinant Naa10 was able to acetylate peptides corresponding to published lysine substrates (11-13). These peptides were approximately 20 residues long, and contained the reported lysine in the center of the peptide. We assayed three reported substrates: MLCK, MSRA and Runx2, and a peptide corresponding to the N-terminus of actin, a known Naa10 substrate, as a positive control (9,10). We carried out acetyltransferase assays with a saturating concentration of peptide (1 mM) and radiolabeled acetyl-CoA and measured the signal by scintillation counting. While Naa10 robustly acetylated its canonical actin substrate, there was no difference in the levels of acetylation in the three substrates in the absence or presence of Naa10 (**Figure 2A**). These experiments reveal that Naa10 does not contribute to the acetylation of MLCK, MSRA and Runx2 lysine peptide substrates.

Naa10 does not acetylate lysine residues within MSRA and RUNX2 proteins—To address the possibility that an expanded recognition motif on the substrates might be required for acetylation by Naa10, we subsequently performed the acetylation reactions using intact protein substrates or domains. To do this, we recombinantly expressed the Runt domain from Runx2, and full length MSRA. We incubated the proteins or actin peptide (50 uM) with radiolabeled acetyl-CoA in the presence and absence of Naa10. After allowing the reaction to take place for 4 hours, we added trichloroacetic acid (TCA) to precipitate the protein and quantified acetylated protein in the pellet by scintillation counting. Once again, we did not observe any difference in the level of acetylation of the substrates with or without Naa10 present, in contrast to the acetylation of actin, which showed enzyme dependence (**Figure 2B**). We note that the difference in the counts for the actin control in figure 2 are due to the use of different substrate concentrations, 1 mM and 50 uM, used for the experiments represented in panels 2A and 2B, respectively. The different concentrations reflect the nature of the experiments done.

To evaluate the nature of acetylation of the substrates that was observed, we carried out reactions with increasing concentrations of acetyl-CoA in the presence and absence of Naa10. This analysis revealed that while Naa10 displayed Michaelis-Menten kinetics against its actin substrate (**Figure 3A**), it showed first order kinetics against Runx2 and MSRA (**Figures 3B and 3C**). When Naa10 was omitted from the reaction, the acetylation against actin was negligible, while the acetylation of Runx2 and MSRA did not change (**Figure 3**). This data strongly suggests that the acetylation of Runx2 and MSRA are independent of Naa10, and are therefore not enzymatic events, but instead are chemical events.

To perform an orthogonal assay for potential Naa10-mediated lysine acetylation, we also performed a Western blot with two different antibodies that recognize acetylated lysines (Cell Signaling – Cat No. 9681 and ImmuneChem – Cat No. ICP0380). In the previously reported studies claiming Runx2 and MSRA acetylation by Naa10, the antibody from Cell Signaling was employed (11,12). We also used the same buffer conditions in the assay that were reported in the prior studies. Since there is no known lysine substrate of Naa10, we used acetylation of the histone tetramer H3/H4 by hMOF as a positive control, as this is a well-studied enzyme-dependent lysine acetylation event (23). In agreement with the radioactive assays, there was no difference in the signal between lysine acetylation whether Naa10 was present or not for both Runx2 and MSRA (**Figure 4**), confirming that the larger intact protein substrates or domains could not be acetylated by Naa10. In fact, the Cell Signaling antibody detected a signal in the samples without any acetyl-CoA added, suggesting that the Runx2 and MSRA proteins may undergo chemical acetylation in the bacterial cells used for expression. This contrasts with H4, which was only acetylated in the presence of both hMOF and acetyl-CoA (**Figure 4**).

Naa10 in the context of the NatA complex cannot acetylate lysine residues—To address the possibility that Naa10 requires assembly into the Naa10/Naa15 NatA complex for acetylating lysine substrates, we prepared a recombinant human NatA complex in insect cells and assayed its ability to acetylate lysine residues in the context of peptide and protein substrates. As shown in **Figure 5**, these results are identical to those with Naa10, indicating that Naa10 in the NatA complex is unable to acetylate Runx2 and MSRA.

Naa10 and NatA do not interact with MRSA and Runx2— Previous reports have claimed that Naa10 directly interacts with MRSA and Runx2 (11,12). To address the possibility that Naa10 or NatA may at least interact with the putative lysine containing substrates in MRSA and Runx2, we carried out pulldown studies. We performed the pulldown by using GST-tagged MSRA and Runx2. In contrast to previous reports, we were unable to detect an interaction between Naa10 and either substrate (**Figure 6A**). Similar studies with the NatA complex also failed to show an interaction. Specifically, while Naa50 successfully pulled down NatA as previously reported (26), MSRA and Runx2 did not (**Figure 6B-C**). Taken together, these studies failed to detect a direct interaction of Naa10 and NatA with MRSA or Runx2.

Discussion

Acetylation has long been recognized as an important protein modification (27). Although

much of the early research examined its role as a histone modification (1), studies have found that thousands of non-histone proteins are acetylated in cells, showing that the modification is much more widespread than initially thought (3). Despite the expansion in the number of lysines targeted for acetylation, the enzymes responsible for, and the functional consequences of the majority of these marks remains unknown.

The data presented here suggest that the acetylation of Runx2 and MSRA is not an enzymatic event, but rather a chemical one. As a moderately reactive high-energy acetyl-donor, acetyl-CoA can acetylate lysines in the absence of an enzyme under the right conditions. This is particularly true if the lysine is located in close proximity to other basic residues, which lowers the pKa of the epsilon amino group to allow for a nucleophilic attack on the acetyl group (28,29). Indeed, the lysines reported for both Runx2 and MSRA cluster within a basic patch on the proteins (30,31) (**Figure 7**).

The results presented here are in contrast to previous reports claiming that Naa10 is able to directly interact with and acetylate lysine residues within several protein targets including Runx2, MSRA, and MLCK (11-13). This discrepancy can be explained by a number of factors. The authors of the previous studies generated knockouts of Naa10 in mice and tissue culture for many of the assays performed. However, as the NatA complex targets hundreds of proteins for acetylation, pleiotropic effects can occur upon depletion or overexpression of the protein, the causes for which can be difficult to decipher. This is reflected in the diverse phenotypes found in NatA null yeast (7). In addition, immunoprecipitation from cells cannot distinguish between direct and indirect protein-protein interactions so given that Naa10 has multiple N-terminal substrates in cells, it is likely that the observed Naa10 interactions in cells were not direct. Therefore, the *in vivo* and cell-based results must be interpreted with caution. In the *in vitro* system described here, neither Naa10 alone nor Naa10 in the context of the NatA complex is observed to acetylate or interact with these putative lysine substrates. Instead, we found that these substrates are chemically acetylated. Chemical acetylation of lysines has been shown to be an important modification in its own right, and it may be significant for regulating these proteins as well.

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Author Contributions: R.S.M. and Z.M.M. performed the experiments described in the manuscript. R.S.M. and Z.M.M. prepared manuscript figures and text. R.M. designed and supervised experiments by R.S.M. and Z.M.M. and revised manuscript text and advised on figure presentation. All authors read and approved the submitted manuscript.

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The abbreviations used are: NAT, N-terminal acetyltransferase; MSRA, methionine sulfoxide reductase A; MLCK, myosin light chain kinase; TEV, tobacco etch virus; OD, optical density

FIGURE LEGENDS

Figure 1. Structure of NATs is incompatible with lysine acetylation. (A) Superposition of the Gcn5 KAT/histone H3 peptide complex (dark and light blue respectively) with Naa10 N-terminal peptide complex (yellow and wheat respectively). The proteins are shown as cartoons and the peptide ligands are shown as sticks. Naa10 is shown with its β6-β7 loop in orange (B) Superposition of NAT/N-terminal peptide complexes with Naa10 (yellow), Naa40p (salmon) Naa50p (green), and the NAT from *Sulfolobus solfataricus* (ssNAT- purple).

Figure 2. Naa10 does not acetylate peptides or proteins corresponding to reported lysine substrates. Recombinant human Naa10 is used for these experiments. (A) Radioactive filter binding assay of peptide acetylation in the presence or absence of Naa10. An N-terminal actin peptide is used as a positive control. (B) Radioactive TCA precipitation assay of protein acetylation with Runx2 and MSRA in the presence of absence of Naa10. Experiments were carried out in triplicate and error bars are indicated.

Figure 3. The kinetic profiles of Naa10 against MSRA and Runx2 are indicative of chemical acetylation. Radioactive filter assay against (A) N-terminal actin peptide, (B) MSRA, and (C) Runx2 at increasing concentrations of acetyl-CoA in the presence (circles) and absence (squares) of Naa10. Experiments were carried out in triplicate and error bars are indicated.

Figure 4. Western blot detection of acetylated MSRA and Runx2 shows independence of Naa10. Western blots are shown using two different antibodies from (A) Cell signaling (#9681) and (B) ImmuneChem (ICP0380) in the absence or presence of acetyl-CoA and Naa10 with MSRA and Runx2 as substrates. The hMOF KAT with histone H3/H4 substrate is used as a positive control. Coomassie staining of the gel is shown below the western blots.

Figure 5. NatA does not acetylate peptides or proteins corresponding to reported lysine substrates. Recombinant human Naa10/Naa15 (NatA) was used for these experiments. (A) Radioactive filter binding assay of peptide acetylation in the presence or absence of Naa10. An N-terminal SASE peptide is used as a positive control. (B) Radioactive TCA precipitation assay of protein acetylation with Runx2 and MSRA in the presence of absence of Naa10. Experiments were carried out in triplicate and error bars are indicated.

Figure 6. Naa10 and NatA do not interact with MSRA or Runx2. (A) GST pulldown assays of Naa10 using GST tagged Runx2 and MSRA. Coomassie staining of SDS-PAGE are shown for both the input (left) and elution (right). Corresponding migrating proteins in the gels are labeled. (B) GST pulldown assays of Naa10/Naa15 (NatA) using GST tagged Runx2 and MSRA. Carried out as described in A. (C) As a positive control, pulldowns of NatA were carried out using GST tagged Naa50. For all panels asterisks indicate free hydrolyzed GST.

Figure 7. The lysines in MSRA and Runx2 are found in proximity to basic residues that could facilitate chemical acetylation. Crystal structures of (A) bovine MSRA and (B) murine Runx1 (90% identity to Runx2) highlighting the lysines within these proteins reported to be acetylated by Naa10 (yellow) as well as proximal basic residues (salmon).

Figure 1

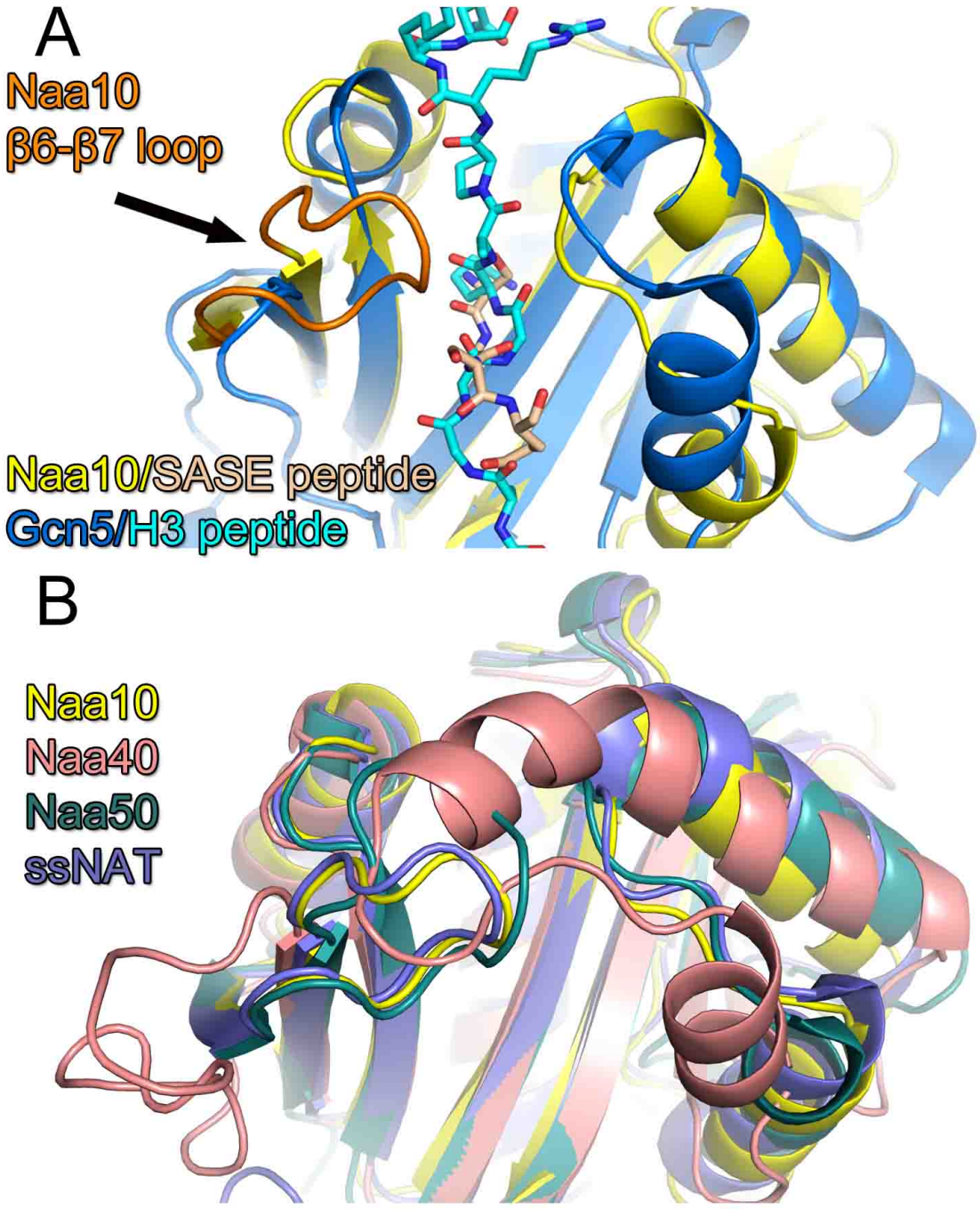


Figure 2

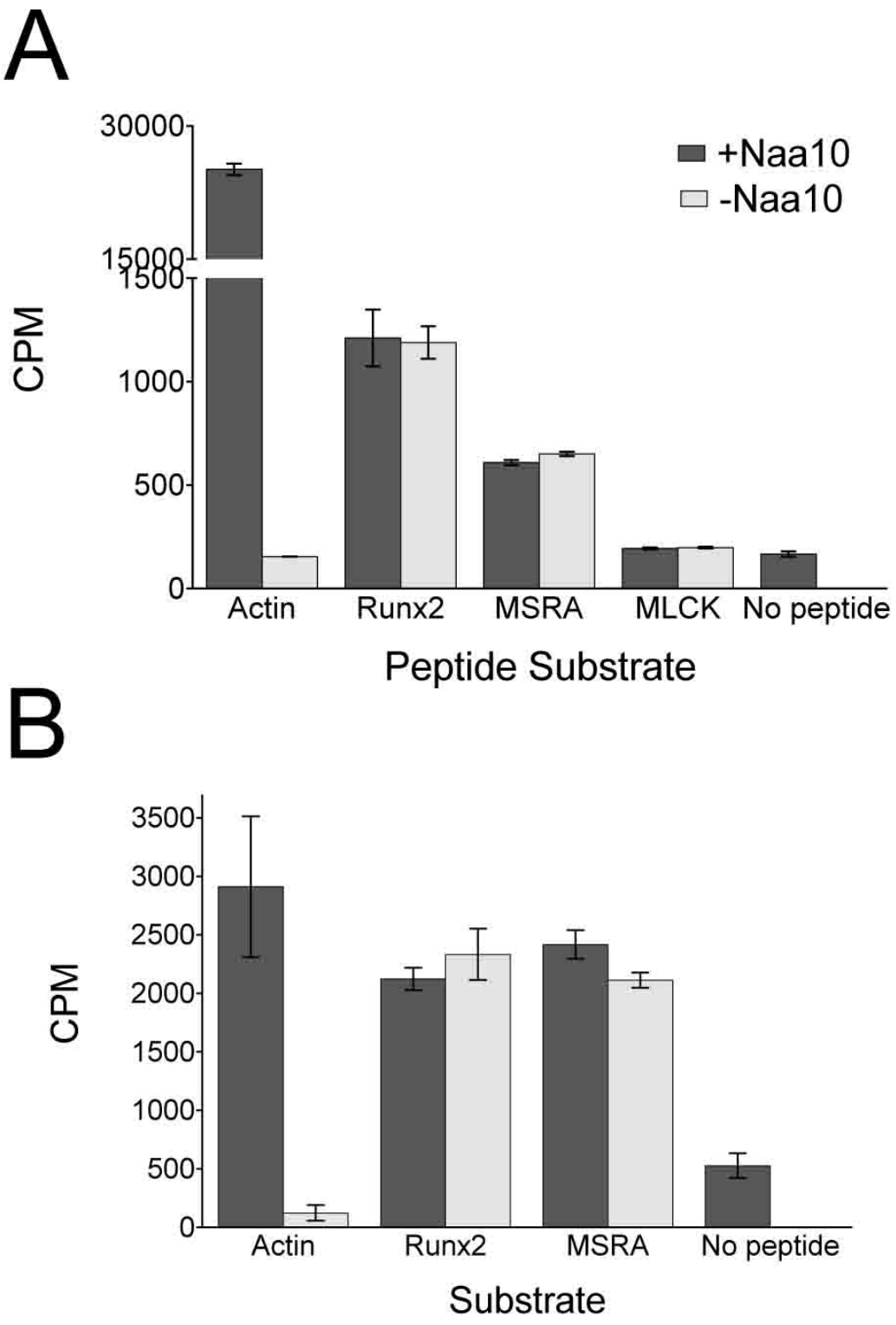


Figure 3

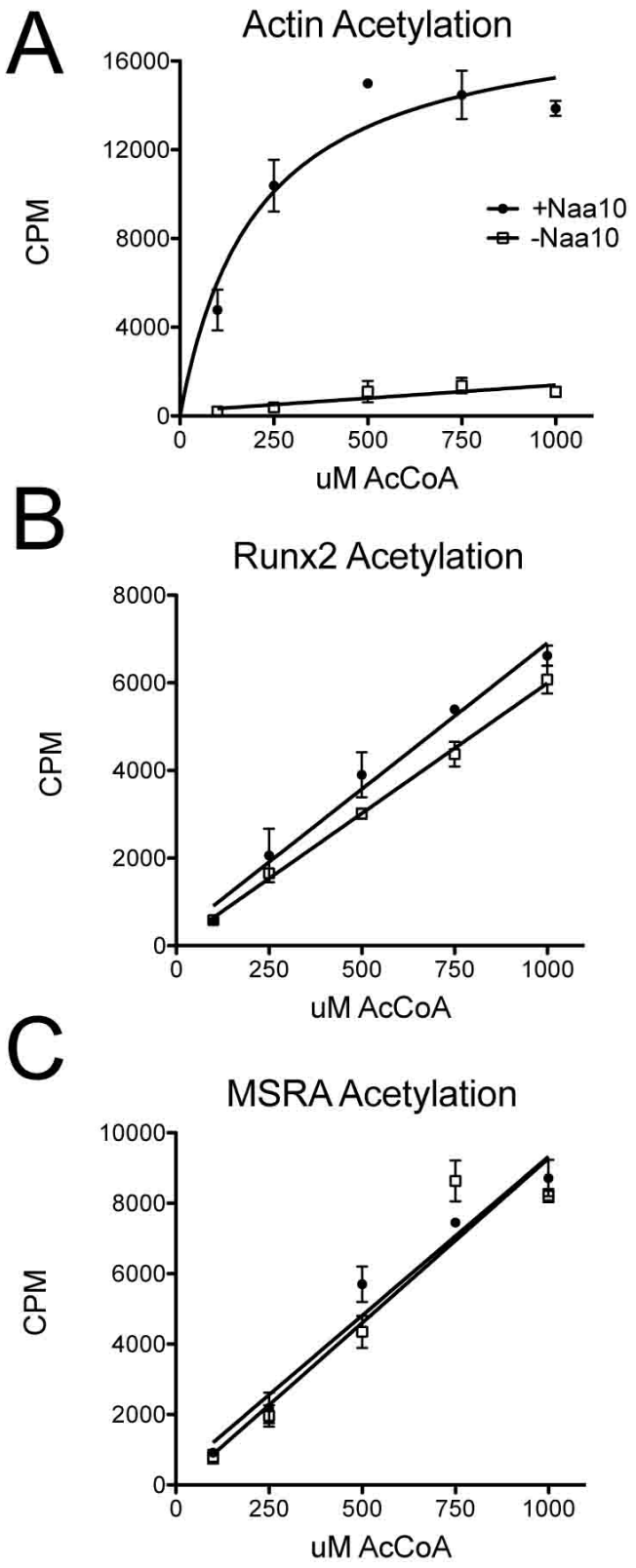


Figure 4

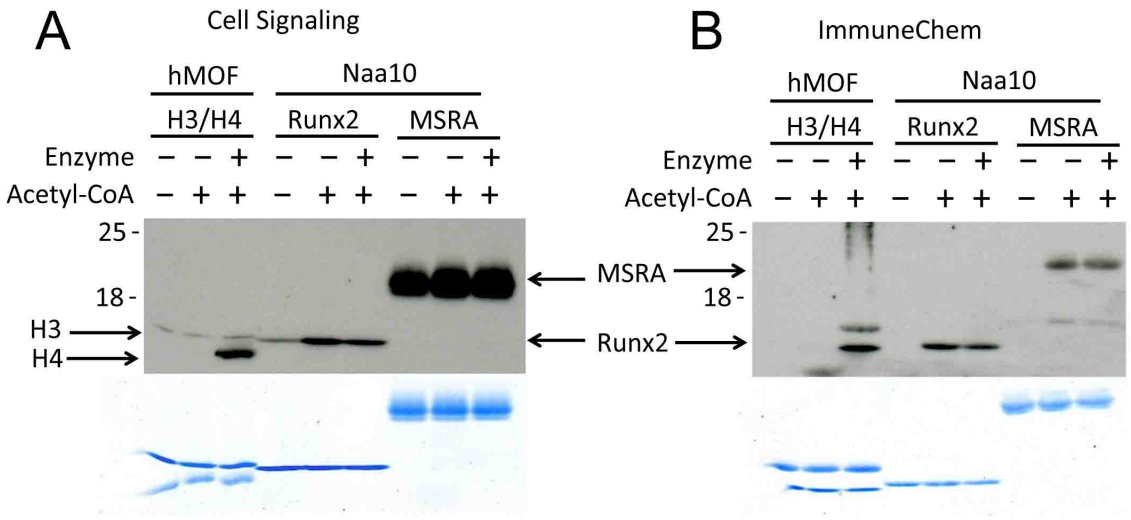


Figure 5

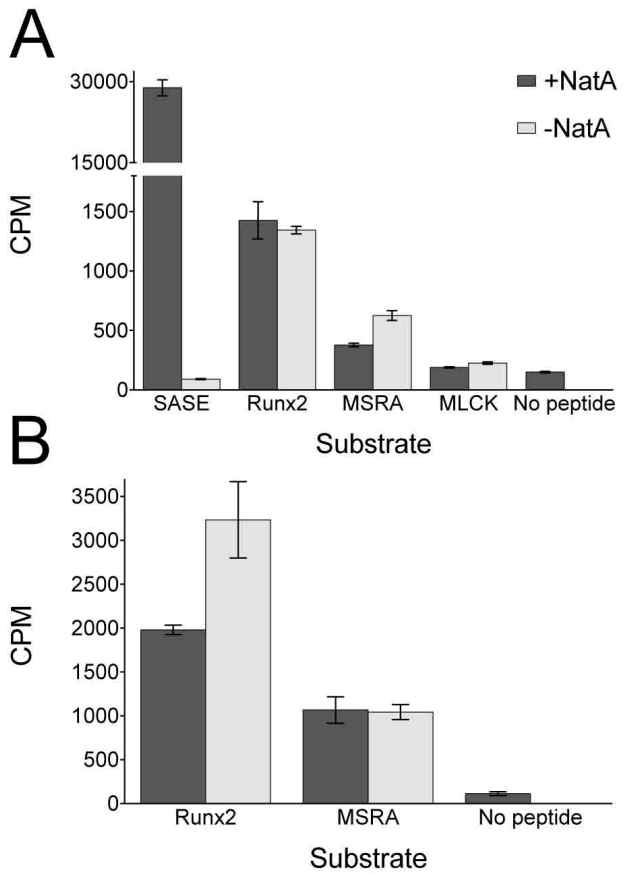


Figure 6

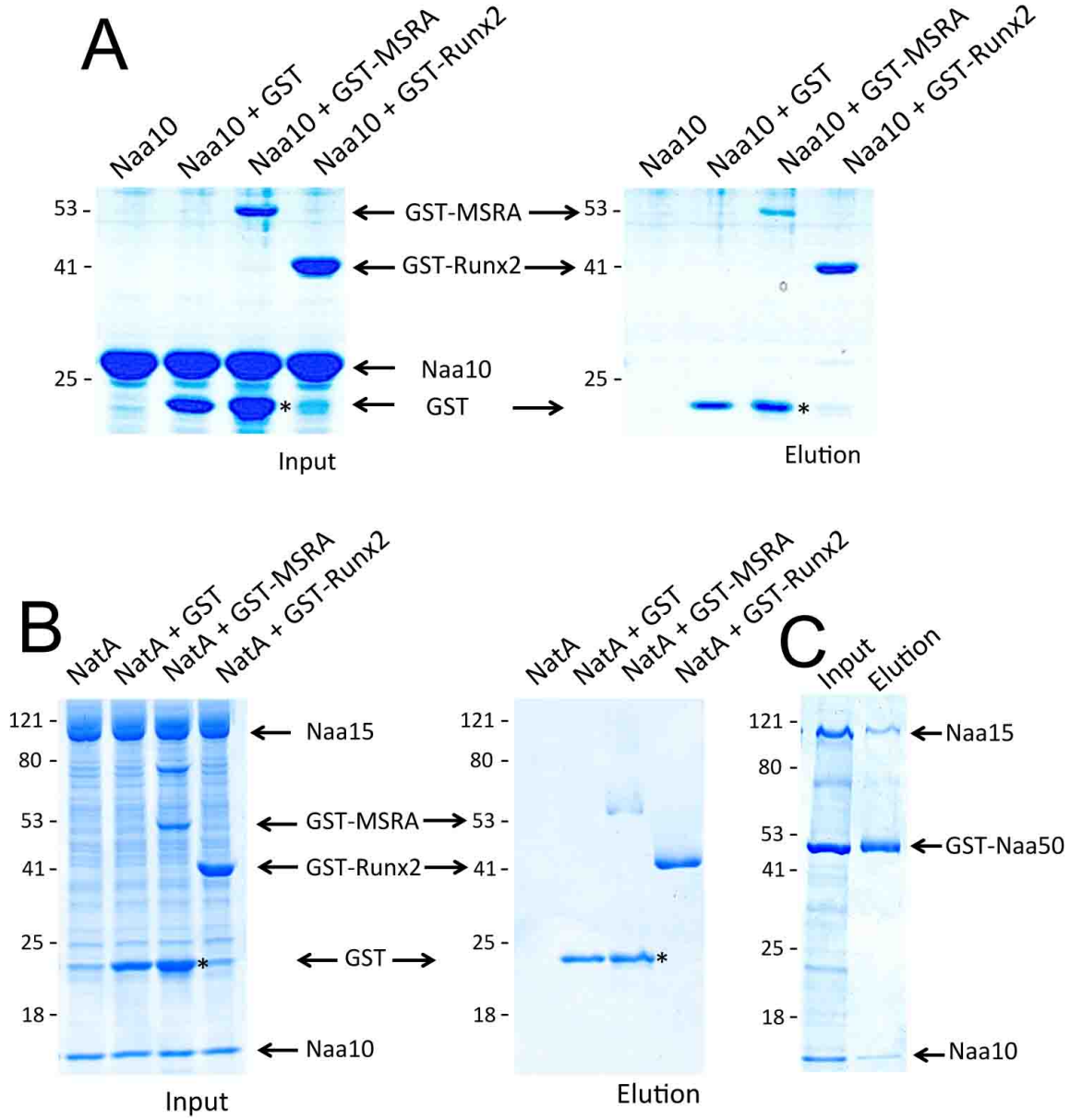
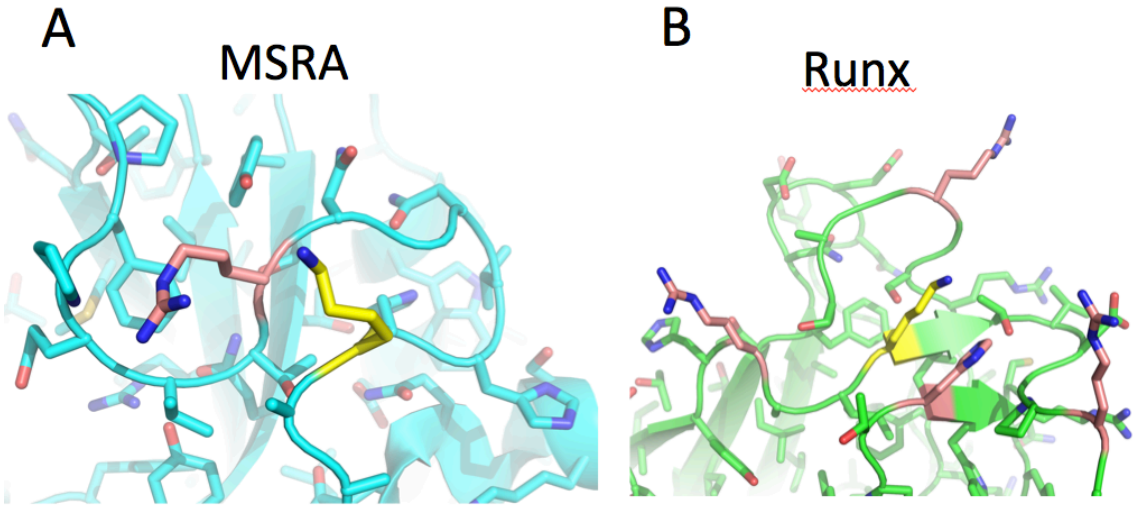


Figure 7



The N-terminal acetyltransferase Naa10/ARD1 does not acetylate lysine residues

Robert S. Magin, Zachary M. March and Ronen Marmorstein

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