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PHOSPHORYLATION OF MSI-1 IS REQUIRED FOR FORGETTING IN CAENORHABDITIS ELEGANS

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ABSTRACT

The Musashi family of RNA-binding proteins controls several biological processes including stem cell maintenance, cell division, and neural function. Previously, we demonstrated that the *C. elegans* Musashi ortholog, *msi-1*, regulates forgetting via translational repression of the Arp2/3 actin-branching complex. However, the mechanisms controlling MSI-1 activity during the regulation of forgetting are currently unknown. Here we investigated the effects of post-translational modifications on MSI-1 activity. We show that MSI-1 function is likely controlled by alterations of its activity rather than its expression levels. Furthermore, we found that MSI-1 is phosphorylated and identified the phosphorylation sites in the worm MSI-1 using mass spectrometry. Moreover, CRISPR-based manipulations of MSI-1 phosphorylation sites revealed that phosphorylation is necessary for MSI-1 function in both short- and long-term memory. Thus, our study provides a first insight into the controlling mechanism of MSI-1 activity during forgetting and may facilitate the development of novel therapeutic approaches.

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ABBREVIATIONS

AMPA-type glutamate receptor	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor
DA	diacetyl (2,3-butanedione)
DAMB	dopamine receptor expressed in mushroom bodies
GLR-1	GLUTAMATE RECEPTOR-1 (protein, *C. elegans*)
LTAM	long-term associative memory
msi-1	*musashi-1 (*gene, *C. elegans)*
MSI-1	MUSASHI-1 (protein, *C. elegans*)
NOTCH	neurogenic locus notch homolog proteins
RBP	RNA-binding protein
RRM	RNA-recognition motif
STAM	short-term associative memory
TGF-β	transfroming growth factor beta
WNT	portmanteau created from the names Wingless and Int-1

1 INTRODUCTION

Characterization of molecules and mechanisms driving learning and memory is crucial to better understand the regulation of cognitive processes. Furthermore, studies investigating the molecular mechanisms of memory acquisition, consolidation and retrieval may provide new therapeutically relevant directions to treat diseases related to altered memory function (de Quervain, Schwabe, & Roozendaal, 2017; Hyman, 2008; Onyike, 2016).

A plastic neural system requires not only the acquisition but also the elimination of memories for its proper function (McGaugh, 2000). Accumulating evidence supports the existence of several parallel-acting intrinsic forgetting pathways that regulate various stages of memory processing. For instance, in the *Drosophila* mushroom body activation of Rac1 and Cdc42, members of the RhoGTPase family, increases forgetting of differentially consolidated memories (Gao et al., 2019). On the other hand, the dopamine receptor DAMB mediates forgetting via Gq activation and subsequent Ca^{2+} signaling mobilization (Himmelreich et al., 2017). RhoGTPases are involved in memory decay during object recognition in mice (Liu et al., 2016) and hippocampal dopamine signaling appears to diminish the late consolidation of cocaine-associated memories in rodents (Kramar, Chefer, Wise, Medina, & Barbano, 2014). Furthermore, mouse Synaptotagmin-3 is responsible for AMPA-type glutamate receptor removal from the synaptic membrane, which induces long-term depression and as a consequence, elimination of spatial memories (Awasthi et al., 2019). In *C. elegans*, TIR-1/JNK-1 pathway mutants display accelerated forgetting of olfactory and gustatory memories (Inoue et al., 2013). We previously showed that the *musashi* gene ortholog, *msi-1*, actively promotes forgetting in *C. elegans*, via the translational repression of members of the Arp2/3 actin branching complex, thus, altering the actin cytoskeleton structure and synapse size

(Hadziselimovic et al., 2014). Altogether, these previous results highlight the presence of conserved complexes and controlled mechanisms that regulate forgetting.

The *musashi* gene was originally identified as a regulator of asymmetric cell division in *Drosophila* (Okano et al., 2005). Musashi family members are RNA-binding proteins that interact with the 3`UTR region of target RNAs in a sequence-specific manner (Iwaoka et al., 2017). The Musashi structure consists of two tandem positioned RNA-recognition motifs (RRMs) located at the N-terminal region of the molecule and a putative disordered region at the C-terminal end (Iwaoka et al., 2017; Sakakibara et al., 2002). Both the domain structure as well as the amino acid sequence of the RRMs are evolutionarily highly conserved throughout the members of the Musashi family in different species. In vertebrates, the two Musashi members, MSI1 and MSI2, are predominantly expressed in the developing and adult nervous system (Sakakibara et al., 2002). Several studies suggest that Musashi proteins contribute to embryonic development and maintenance of stem cell properties by regulating components of multiple signaling pathways, including WNT (Spears & Neufeld, 2011), NOTCH (Imai et al., 2001), and TGFβ (Kudinov et al., 2016). In order to fulfill these diverse cellular functions, the expression levels of MSI must be tightly controlled (Hentze, Castello, Schwarzl, & Preiss, 2018; Muller-McNicoll, Rossbach, Hui, & Medenbach, 2019). Accordingly, several studies have aimed to identify key regulators of Musashi expression, for example, in human HPSCs (Belew et al., 2018) and during mammalian spermatogenesis (Sutherland et al., 2014). Beside the regulation of MSI activity at the transcriptional and translational levels, post-translational modulation could also have a key role in the maintenance of protein homeostasis and activity. Several studies have investigated the function of post-translational modifications of Musashi orthologs. For instance, human MSI2 protein is ubiquitinated in breast cancer cells, resulting in its proteasomal degradation (Choi et al., 2017).

Other studies investigated the role of phosphorylation of MSI1 and MSI2 in *Xenopus* oocytes and showed that phosphorylation is required for the protein's function in cell-cycle regulation (Arumugam, MacNicol, Wang, et al., 2012; A. M. MacNicol, Hardy, Spencer, & MacNicol, 2015). MSI2 undergoes progesterone-dependent phosphorylation during the maturation of *Xenopus* oocytes, which is necessary for its function in translational control. Furthermore, an alternatively spliced MSI2 isoform that lacks the exon containing the phosphorylated residues fails to regulate translation of target mRNAs, suggesting that phosphorylation likely plays an important role in MSI2 function (M. C. MacNicol et al., 2017). These findings highlight the importance of MSI2 phosphorylation and raise the possibility that Musashi activity may be regulated via phosphorylation in most species. Despite the apparent importance of post-translational modifications that may alter MSI-1 affinity to other interacting proteins, its downstream target RNAs or the MSI-1 protein levels through translational auto-regulation (Arumugam, Macnicol, & Macnicol, 2012), their possible role during forgetting has not yet been investigated.

In this study, we show that the function of MSI-1 protein in forgetting is likely modulated by alterations in its activity rather than its abundance. Using mass spectrometry, we identified MSI-1 phosphorylation at amino acid residues T18, S19 and S34. To study the role of phosphorylation in forgetting, we converted the identified sites alone or in combination to alanine, thereby, preventing phosphorylation, and tested the mutant worms for short- (STAM) and long-term (LTAM) associative memory performance. Our findings indicate that both the single and the simultaneous T/S to A mutations inhibit forgetting to a similar extent as observed for the *msi-1* deletion mutant, suggesting that the phosphorylation is essential for MSI-1 function. The phospho-mimicking mutation, *msi-1(S34D),* on the other hand, does not interfere with memory. Interestingly, *msi-1(T18D)* and *msi-1(S19D)* mutations impaired short-term but not long-term forgetting. This

suggests that emulating constitutive phosphorylation at specific residues may also impair MSI-1 function during short-term memory. Altogether, we identified specific residues at the N-terminal end of *C. elegans* MSI-1 proteins that are phosphorylated and demonstrated that phosphorylation of these sites is necessary for the protein activity during forgetting.

2 MATERIALS AND METHODS

2.1 General methods and *C. elegans* strains used

Standard methods were applied for maintaining and manipulating *C. elegans* (Brenner, 1974). The *C. elegans* Bristol strain, variety N2, was used as wild-type reference in all experiments. To purify plasmids for microinjection we used midiprep plasmid purification kit (Qiagen, Hilden, Germany). Extrachromosomal array expressing transgenic lines were generated by injecting DNA at a concentration of 10-100 ng/µl into both arms of the syncytial gonad of young adult worms as described previously (Mello, Kramer, Stinchcomb, & Ambros, 1991). *sur-5P::mDsRed2* or *myo-2P::mCherry* were used as transformation markers at 10 or 2.5 ng/µl concentration respectively. Chromosomal integration of extrachromosomal arrays was done by UV irradiation (Mariol, Walter, Bellemin, & Gieseler, 2013). Genome editing of the target gene loci was performed using co-CRISPR/Cas9 strategy as described previously (Arribere et al., 2014). For detailed description, see Genome editing with CRISPR-Cas9. All generated strains were four times backcrossed with the wild-type strain. The *C. elegans* strains used in this study are: *msi-1(os1); utrIs3[pmsi-1::msi-1(cDNA)::1xMYC::msi-1(3'UTR), sur-5P::mDsRed2, myo-3P::mCherry]*, *msi-1(os1); utrIs17[pmsi-1::msi-1(cDNA)::3xFLAG::msi-1(3'UTR), myo-2P::mCherry]*, *msi-1(utr12[T18A,S19A,S34A*)], *msi-1(utr11[T18A,S19A])*, *msi-1(utr17[T18A])*, *msi-1(utr18[S19A])*, *msi-1(utr7[S34A])*, *msi-1(utr46[T18D]), msi-1(utr43[S19D])*, *msi-1(utr16[S34D])*.

2.2 Genome editing with CRISPR-Cas9

Modification of the endogenous loci was performed using Co-CRISPR/Cas9 genome editing method described previously (Kim et al., 2014). sgRNA was designed to direct Cas9 cleavage at the desired locus using ApE plasmid editor software based on the previously published sequence requirements (Arribere et al., 2014). For Co-CRISPR genome editing, sgRNA targeting the locus

of interest, sgRNA targeting the *dpy-10* locus, repair oligonucleotides (Microsynth AG, Balgach, Switzerland) for *dpy-10* and for *msi-1* with the desired modifications and a plasmid allowing expression of Cas9 in the germline were co-injected into the gonad of young adult worms. F1 animals showing roller phenotype were singled and allowed to propagate. To confirm the presence of CRISPR-Cas9-initiated modifications, worms were genotyped with PCR using the following primers: 5'-CAGCAGAAGCAGCAGCATCAG-3' and 5'-TGTGAGAAGTAAAAACGGAGCAAAC-3'. The primers amplify a 500 base pair long DNA from the *msi-1* locus. Amplified PCR products were subjected to digestion with SalI enzyme (New England Biolabs, Ipswich, MA) resulting in two smaller fragments if the CRISPR/Cas9-modified T18/S19 alleles were present. Similarly, the amplified PCR products were digested with HpyCH4V or MseI enzymes (New England Biolabs, Ipswich, MA) to detect the presence of *msi-(S34A)* or *msi-1(S34D)* alleles, respectively. The genotype of the animals was confirmed with sequencing (Microsynth AG, Balgach, Switzerland). Homozygous animals carrying the modified allele were backcrossed four times.

2.3 Chemotaxis assays

Chemotaxis to diacetyl was investigated in synchronized young adult populations as previously described (Bargmann, Hartwieg, & Horvitz, 1993). Briefly, worms were washed three times with CTX solution (5 mM KH_2PO_4/K_2HPO_4 pH 6.0, 1 mM $CaCl_2$, and 1 mM $MgSO_4$) and approximately 80-150 worms were placed in the middle of a 10 cm CTX test plate (1.9% agar, 5 mM KH_2PO_4/K_2HPO_4 pH 6.0, 1 mM $CaCl_2$, and 1 mM $MgSO_4$). Worms were given a choice between a spot of 0.1 % diacetyl (Sigma Aldrich, St. Louis, MO) diluted in ethanol versus a control spot of ethanol. Additionally, 1µl of 20mM sodium azide was used to paralyze the worms that reached the chosen spot. The distribution of the worms on the testing plate was determined after 1

hour and the chemotaxis index (number of worms in the diacetyl spot minus number of worms in the ethanol spot divided by the total number of worms on the plate) was calculated as described previously (Bargmann et al., 1993). Short-term or long-term aversive olfactory conditioning was performed as previously described (Nuttley, Atkinson-Leadbeater, & Van Der Kooy, 2002; Vukojevic et al., 2012). Briefly, for the short-term assays, worms were subjected to starvation for 1 hour in the presence of 2µl diacetyl on 10 cm CTX plates. Following training chemotaxis of the worms towards diacetyl was assessed directly or after a 1-hour resting period. Long-term memory consolidation was induced with 2 times repeated 1-hour training session with a 30 minutes rest in presence of food in between. Following conditioning, worms were kept on NGM plates seeded with OP_{50} bacteria for 24 hours and tested for diacetyl chemotaxis after the recovery phase.

2.4 Protein extraction and immunoprecipitation

For immunoprecipitation, worms were collected in ice-cold RIPA buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 1% Triton-X-100, 0.5% sodium-deoxycholate, 0.1% SDS, 1mM EDTA, 10 mM NaF, 1 mM Na-orthovanadate) supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland). Samples were homogenized in Mixer Mill MM 301 (Retsch GmbH, Germany) for 30s repeated four times. Lysates were cleaned by centrifugation at 13.000 rpm for 20 min at 4°C. Protein concentration of the supernatant was measured using Pierce BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions. Anti-FLAG M2 Affinity Gel (Sigma Aldrich, St. Louis, MI) and Anti-c-Myc Agarose Affinity Gel (Sigma Aldrich, St. Louis, MI) bead-conjugated antibodies were used to overnight immunoprecipitate the protein of interest from 2 mg total worm protein extract. Following incubation, samples were washed 3 times with HNTG buffer (50mM HEPES pH 7.4, 150mM NaCl, 10% glycerol, 1% Triton-X-100).

2.5 Western blot analysis

Samples were subjected to SDS-PAGE, transferred to PVDF membranes, blocked with 5% non-fat dry milk in TBST (50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.05% Tween-20) and incubated with primary antibodies as indicated. Antibodies used were: mouse anti-c-Myc 9E10 (1:1000, Thermo Fisher Scientific, Waltham, MA), mouse anti-FLAG (1:1000, Sigma Aldrich, St. Louis, MI) and mouse anti-actin (1:2000, Merck Millipore, Burlington, MA). Primary antibodies were detected using HRP coupled secondary antibodies (1:5000, Jackson ImmunoResearch Laboratories, Cambridge House, UK). Chemiluminescent signal was developed using Clarity and ClarityMax Western Blotting Substrates (BioRad Laboratories Inc., Hercules, CA) followed by detection with a FujiFilm ImageQuant LAS-4000 detector (GE Healthcare, Chicago, IL).

2.6 Mass spectrometry analysis

For the identification of post-translational modifications of *C. elegans* MSI-1, liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used. Immunoprecipitated protein samples were subjected to reduction (20mM dithiothreitol, 50mM Tris-HCl pH 8.0) and alkylation (50mM iodoacetamide, 50mM Tris-HCl, pH 8.0) followed by overnight tryptic, chymotryptic, AspN and Lys-C (Promega, Madison, WI) digestions. Next, the digested samples were acidified with TFA (1% in water), then desalted using Vydac C18 Silica Microspin columns (5-200µl, 5-60µg, The Nest Group Inc., Southborough, MA), followed by elution with 80% acetonitrile/0.1% TFA solution. Samples were separated using reverse phase liquid chromatography (New Objectives, MA) and the eluted peptides were ionized and analyzed with Orbitrap FT hybrid mass spectrometer attached to LTQ instrument (Thermo Fisher Scientific, Waltham, MA). Resulting MS/MS spectra were evaluated with Proteome Discoverer 1.4 (Thermo Fisher Scientific, Waltham, MA) using Mascot (Matrix Science, London, UK) as search engine.

Mass spectra were annotated using consensus and processing workflows, phosphorylated amino acid residues were identified and the relative abundance of peptides was estimated using Proteome Discoverer 1.4 software (Thermo Fisher Scientific, Waltham, MA). Relative phosphorylation of peptides was calculated as follows:

$$\text{Relative phosphorylation (\%)} = \frac{\text{Abundance (phosphorylated)}}{\text{Abundance (phosphorylated)} + \text{Abundance (non} - \text{phosphorylated)}} \text{ X } 100$$

2.7 Statistical analysis

The behavioral assay results were analyzed and visualized using Prism 5 software (GraphPad Software Inc., San Diego, CA). Main effects and interaction terms were investigated using ANOVA. The p-value threshold was set to nominal significance ($p < 0.05$). Pairwise group comparison was tested using *post hoc* t-tests corrected for multiple comparisons using Bonferroni correction ($pBonf. < 0.05$).

3 RESULTS

3.1 Total MSI-1 abundance does not change upon learning and memory consolidation

Previously, we demonstrated that the C. elegans *msi-1* gene actively regulates forgetting of short- and long-term memories (Hadziselimovic et al., 2014). We hypothesized that C. elegans MSI-1 protein levels could be altered due to changes in gene expression or protein stability upon learning and memory consolidation. To check for possible learning- or memory-related changes in total MSI-1 abundance, we performed a time-course experiment extracting proteins from whole worms carrying an integrated array expressing FLAG-tagged *msi-1* before, right after conditioning and at different time points during the 24-hour recovery. Upon treatments, protein levels were analyzed with western blots and we found that the level of total MSI-1 is constant upon learning and memory consolidation (Fig 1A). To further corroborate these results, total MSI-1 abundance was also estimated using mass spectrometry. For the analysis, FLAG-tagged MSI-1 was immunoprecipitated from lysate of worms collected before, right after or 4 hours after learning. In accordance with the previous result, mass spectrometry also failed to detect a significant learning-induced or memory-related changes in total MSI-1 protein levels upon memory formation or consolidation (Fig 1B). Therefore, MSI-1 activity might be primarily regulated via activity changes mediated by post-translational modifications.

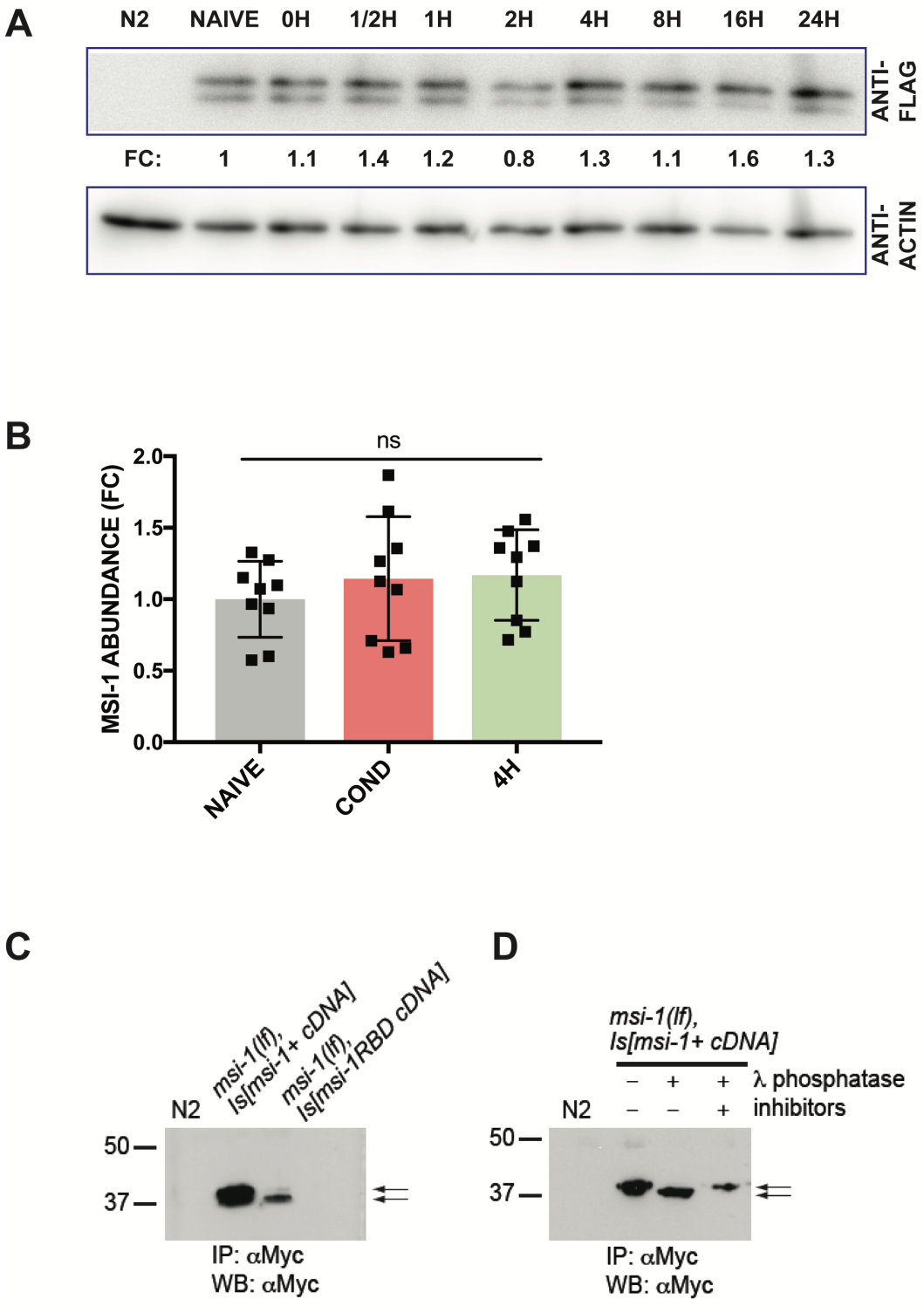

Figure 1. The *C. elegans* MSI-1 is a phosphoprotein with constant abundance upon learning and memory. (A) Worms carrying *msi-1::3xFLAG* multicopy array were subjected to DA-starvation associative learning and 50 worms were tested for protein levels before (NAÏVE), right after (0H) or at different time points followed by conditioning (from 1/2H to 24H). Samples were separated by PAGE, transferred to membranes and probed for FLAG and ACTIN. N2 was used as negative control. Representative western blot is shown. Experiment was repeated 5 times resulting in similar patterns. (B) Worms were collected before (NAÏVE), right after

(COND) or 4 hours after (4H) conditioning, MSI-1 was immunoprecipitated and analyzed with MS. Abundance of MSI-1 was estimated from calculating the summed abundance of all detected MSI-1 peptides resulting from tryptic digestion. Squares represent values as fold changes compared to the mean of NAÏVE. Bars correspond to mean +/- SD. Significance was tested with 1-way ANOVA. ns: not significant. ****=p<0.0001. Graph summarizes results from 3 independent biological replicates, each biological replicate consists of 3 technical repetitions. (C-D) Western blot of total *C. elegans* protein extracted from synchronized adult population, followed by anti-Myc immunoprecipitation, probed with anti-Myc antibody. (C) Detection of Myc-tagged wild-type and RNA-binding mutant MSI-1 protein. (D) Immunoprecipitated Myc-tagged wild-type MSI-1, followed by treatments with or without Lambda phosphatase in absence or presence of phosphatase inhibitor cocktail. Arrows show the two MSI-1 bands with different electrophoretic mobility corresponding to phosphorylated MSI-1 (upper band) and non-phosphorylated MSI-1 (lower band).

3.2 The N-terminal part of MSI-1 is phosphorylated at different residues

Previously, Musashi proteins have been previously classified as phosphoproteins in different organisms (Arumugam, MacNicol, Wang, et al., 2012; A. M. MacNicol et al., 2015; M. C. MacNicol et al., 2017), therefore, phosphorylation could be a possible modulator of MSI-1 activity during forgetting in *C. elegans*. To test this hypothesis, we first explored the phosphorylation status of MSI-1. We analyzed Myc-tagged wild-type and RNA-binding mutant MSI-1 protein immunoprecipitated from *C. elegans* total protein extracts. Using western blot, we found that *C. elegans* MSI-1 migrates as a doublet indicating that the protein might indeed undergo post-translational modifications (Fig 1C, D). Interestingly, the RNA-binding deficient MSI-1 protein shifted to the faster migrating form, suggesting that the modifications might be dependent on the interaction of MSI-1 with target mRNAs (Fig 1C). To confirm that the two bands correspond to phosphorylated and non-phosphorylated forms of MSI-1, the immunoprecipitated MSI-1 protein was treated with lambda phosphatase prior to loading on an SDS-PAGE and western blot analysis. As expected, the phosphatase treatment resulted in a shift of total MSI-1 to the faster migrating form, which was inhibited by the simultaneous presence of phosphatase inhibitors (Fig 1D). Altogether, these results strongly support the hypothesis that MSI-1 is phosphorylated.

3.3 Identification of phosphorylated peptides in the *C. elegans* MSI-1 protein

To identify potential phosphorylation sites of MSI-1, we analyzed the post-translational modifications of the protein using LC-MS/MS. First, *C. elegans* MSI-1 protein was reproducibly detected with high overall coverage (84%) covering most of the potential phosphorylation sites (Fig 2A). Next, we analyzed the results and detected several post-translational modifications including phosphorylated ETSPPVDGHEEAR and LNADSDDGSHGSQDPGK peptides (Table 1).

Peptide	Modification	m/z (Da)
[R].LNADSDDGSHGSQDPGK.[M]	1x Phosphorylation [S34]	890.35
[R].LNADSDDGSHGSQDPGK.[M]	None	850.37
[R].ETSPPVDGHEEAR.[L]	1x Phosphorylation [T18 OR S19]	752.31
[R].ETSPPVDGHEEAR.[L]	None	712.31

Table 1. List of phosphorylated peptides and their non-phosphorylated isoforms from MSI-1 protein detected with mass spectrometry. Summary of the phosphorylated peptides with MS-confirmed phosphorylation and their non-phosphorylated isoforms that were detected as a result of tryptic and Lys-C digestion of MSI-1 protein. The amino acid sequences of the detected peptides are listed in the first column. Amino acids in square brackets represent the location of the enzymatic digestion. Second column highlights modified amino acids and their position in the protein (square brackets). "1x Phosphorylation" refers to detection of single phosphorylation, while "None" indicates absence of post-translational modifications. Third column displays mass-to-charge ratio (m/z) of the detected peptides.

The fragmentation spectrum reproducibly indicated a single phosphorylation of the threonine or serine residues in ETSPPVDGHEEAR peptide, corresponding to either T18 or S19, respectively (Fig 2C), together with the non-phosphorylated peptide counterpart (Fig 2B). Given the overlapping profiles of the alternatively phosphorylated peptide isoforms, explicit differentiation between T18- or S19-phosphorylated ETSPPVDGHEEAR peptides was not possible. In addition, we found a single phosphorylation of the LNADSDDGSHGSQDPGK fragment at the S34 residue (Fig 2E), along with the non-phosphorylated version of the peptide (Fig 2D).

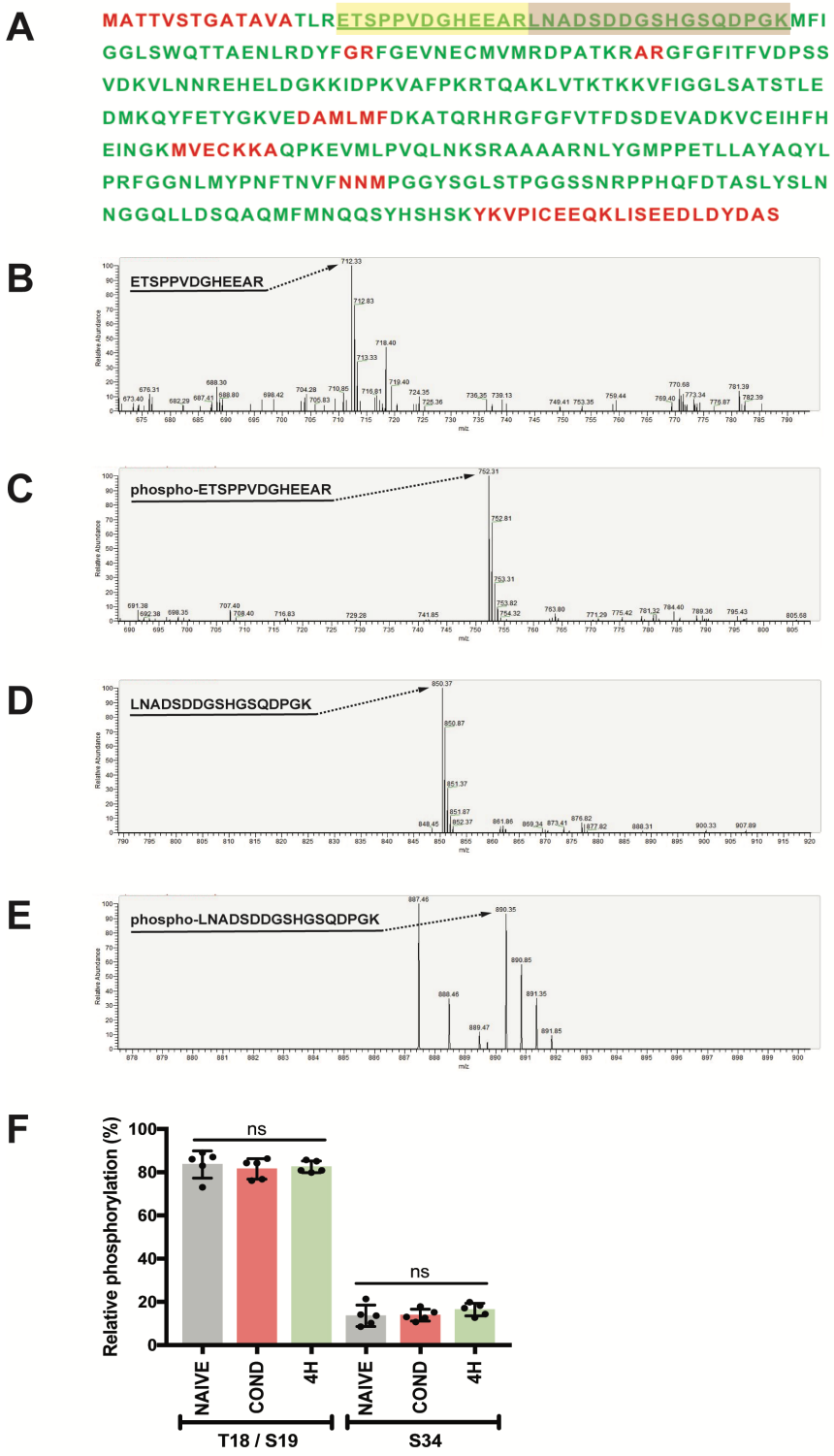

Figure 2. Identification of phosphorylated amino acid residues in *C. elegans* MSI-1. (A) Amino-acid sequence of the full length MSI-1 protein. Residues covered (green), and missing (red) in the mass spectrometry analysis are depicted. Phosphorylated peptides are highlighted: ETSPPVDGHEEAR (yellow) and LNADSDDGSHGSQDPGK (brown). (B-E) Mass spectra of the identified phosphorylated peptides and their corresponding

non-phosphorylated counterparts. Mass spectra shown are captured at the time point corresponding to the apex of the elution peak (based on liquid chromatography) of the given peptide. The mass difference between phosphorylated and non-phosphorylated isoforms matches the m/z value of phosphate (40 Da). (F) Worms were collected before (NAÏVE), right after (COND) or 4 hours after (4H) conditioning, proteins were extracted and MSI-1 was immunoprecipitated. Relative phosphorylation (%) was calculated for each condition (for the details see Materials and methods). Dots represent individual values of relative phosphorylation (%) level of the peptide corresponding to T18/S19 or S34. Bars and whiskers represent mean and SD. Graph summarizes the results from 5 individual biological replicates. Significance was tested using 1-way ANOVA, ns = not significant.

Additionally, mass spectrometry analysis revealed that the ETSPPVDGHEEAR peptide is highly phosphorylated (83.6% ± 6.5), while LNADSDDGSHGSQDPGK peptide phosphorylation is low (13.6% ± 5.0) (Fig 2F) in untrained worms. Finally, we addressed whether there could be learning- or memory-related changes in the phosphorylation state of the detected peptide species. We found that there were no significant detectable changes in the relative phosphorylation of the aforementioned peptides, neither directly after, nor 4 hours after associative learning (Fig 2F). However, our results cannot fully rule out the possibility that cell type-specific or local changes in MSI-1 phosphorylation occur during memory.

3.4 Perturbations in MSI-1 phosphorylation lead to altered memory performance

To study the importance of MSI-1 phosphorylation during memory in *C. elegans*, we introduced various phospho-inhibitory (T/S to A) or phospho-mimetic mutations (T/S to D) in the endogenous protein using CRISPR/Cas9 genome editing (Arribere et al., 2014). None of these mutations affected chemotaxis (Figs 3 and 4). Furthermore, the 1-hour starvation period in the presence of DA evoked decreased chemotaxis towards DA, suggesting that a lack of phosphorylation or an emulated constitutive phosphorylation at the sites in question does not interfere with the learning process (Figs 3 and 4).

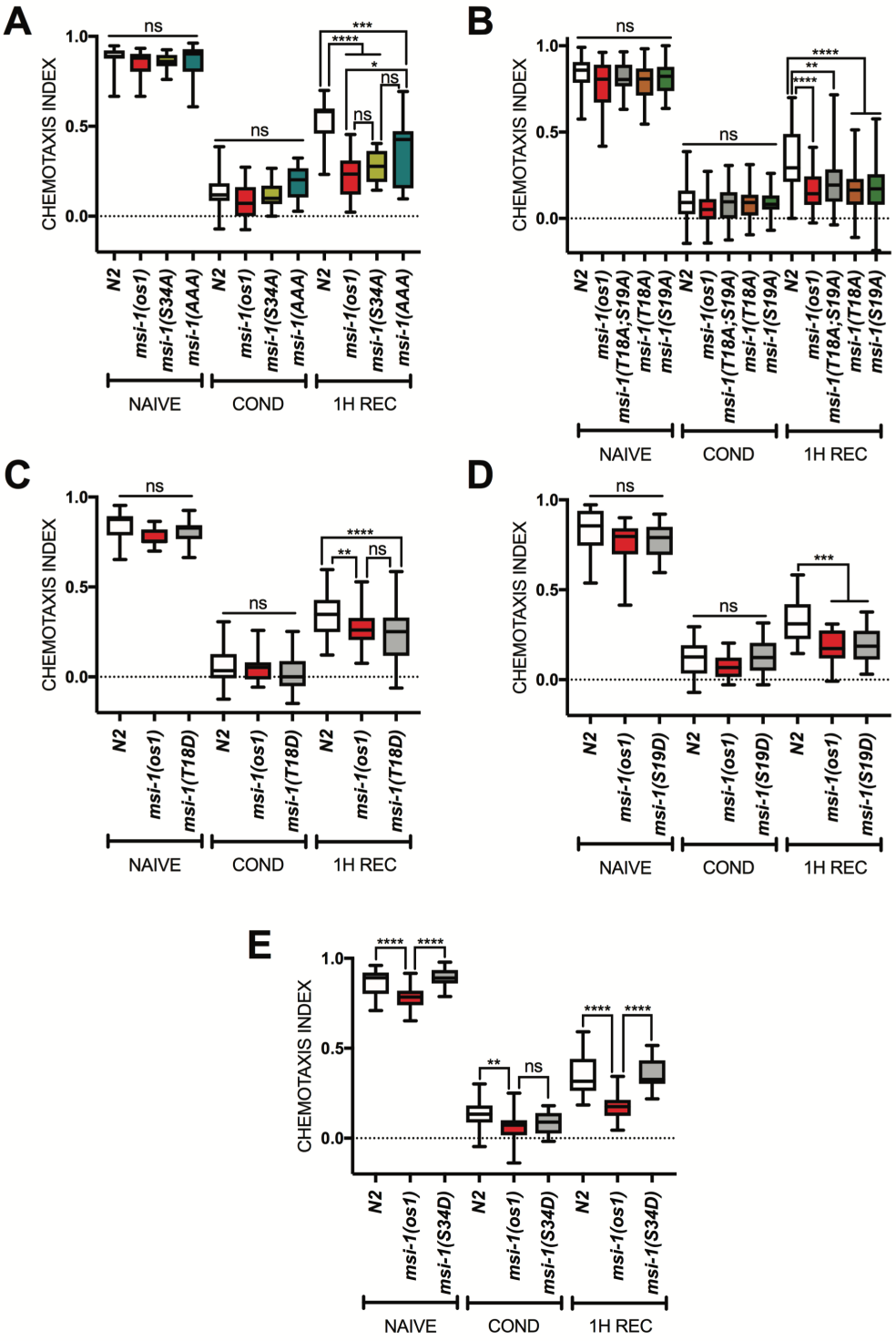

Figure 3. Short-term memory phenotyping of msi-1 phospho-mutants. Negative olfactory STAM was tested in WT, *msi-1(os1)* and animals with phospho-inhibitory (A-B) or phospho-mimetic (C-E) mutations as indicated. Worms were assayed toward 1:1000 diluted DA before (NAÏVE), directly after conditioning in the presence of DA coupled with starvation (COND) or followed by a 1-hour recovery phase (1H REC). All experiments were done in triplicates and repeated at least four times. Data is represented in boxplots with min-max whiskers. Significance was tested with 2-way ANOVA and *post*

hoc t-tests. ns= not significant, asterisks represent Bonferroni-corrected p-values: *=p<0.05, **=p<0.01 ***=p<0.001 and ****=p<0.0001.

To investigate the effects of phospho-inhibitory mutations on memory performance, we assayed the homozygous mutant strains and found that in worms carrying phospho-inhibitory mutations, short-term memory retention was significantly higher compared to wild-type worms and similar to *msi-1* deletion mutant worms (Fig 3A, B). Interestingly, we found that phospho-mimetic mutations of T18D/S19D sites showed a similar phenotype to phospho-inhibitory mutations on short-term memory retention (Fig 3C, D), while *msi-1(S34D)* retained a wild-type STAM (Fig 3E).

Similar to short-term memory, the simultaneous introduction of phospho-inhibitory mutations of T18A, S19A and S34A increased long-term memory retention tested 24 hours after learning similar to *msi-1(os1)* deletion mutant (Fig 4A, B). Furthermore, individual substitution of either S19A or S34A significantly elevated long-term memory retention (Fig 4A, B), while mutating these sites separately to aspartic acid (D) did not interfere with LTAM (Fig 4D, E). Interestingly, the T18A substitution induced an intermediate LTAM phenotype (Fig 4B), laying between wild-type and *msi-1(lf)* mutant memory performance. On the other hand, the T18D conversion resulted only in a very subtle effect on LTAM compared to wild-type worms (Fig 4C). Together, these results show that the phosphorylation state of MSI-1 plays a crucial role in the memory-related function of the protein in *C. elegans* (summarized in Table 2).

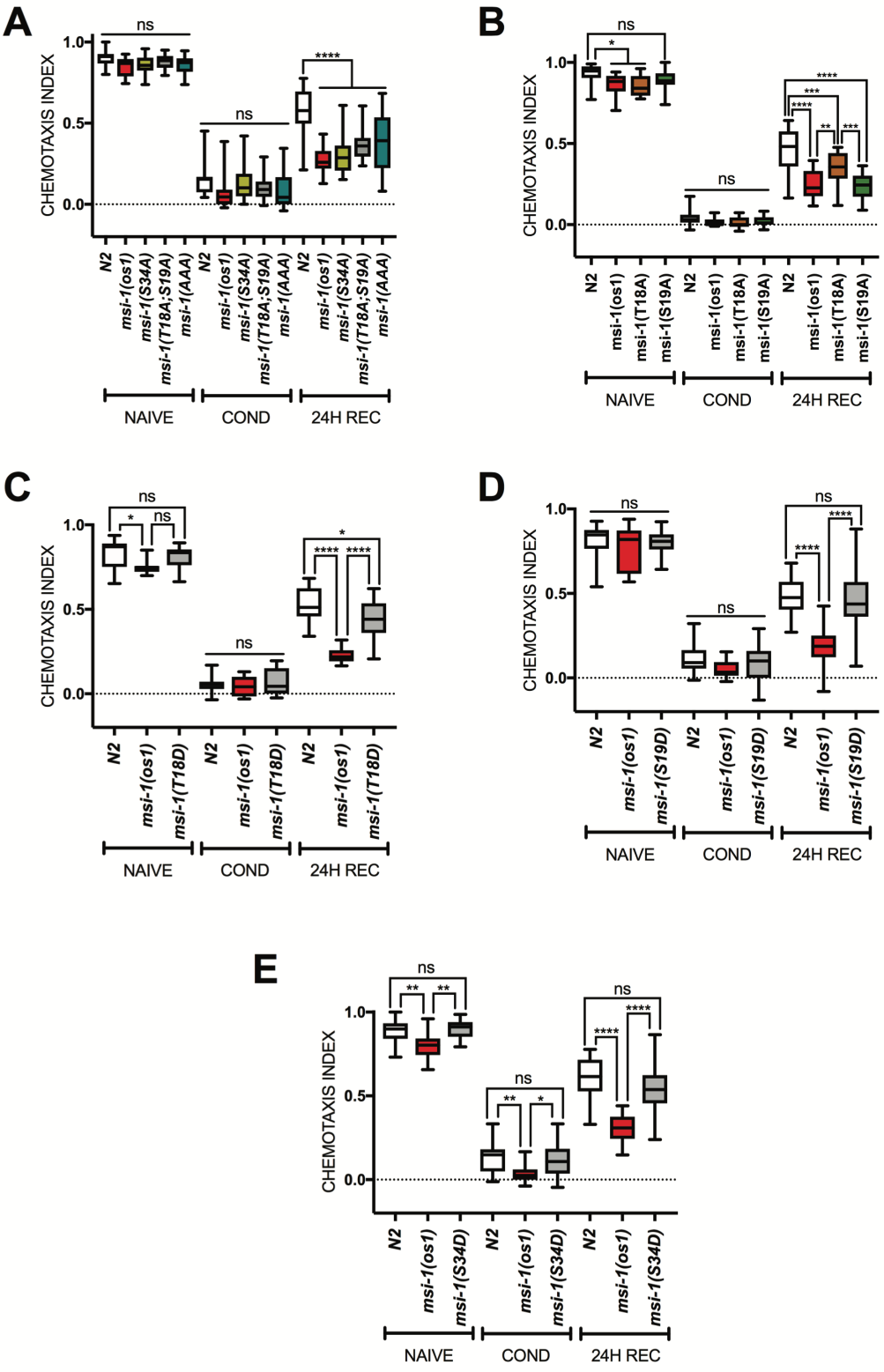

Figure 4. Long-term memory phenotyping of msi-1 phospho-mutants. Negative olfactory LTAM was tested in WT, *msi-1(os1)* and animals with phospho-inhibitory (A-B) or phospho-mimetic (C-E)

mutations as indicated. Worms were assayed toward 1:1000 diluted DA before (NAÏVE), directly after conditioning (COND) or followed by a 24-hour recovery phase (24H REC). All experiments were done in triplicates and repeated at least four times. Data is represented in boxplots with min-max whiskers. Significance was tested with 2-way ANOVA and *post hoc* t-tests. ns= not significant, asterisks represent Bonferroni-corrected p-values: *=p<0.05, **=p<0.01 ***=p<0.001 and ****=p<0.0001.

Genotype	STAM phenotype	LTAM phenotype
os1	Enhanced memory retention	Enhanced memory retention
T18A;S19A;S34A	Enhanced memory retention	Enhanced memory retention
T18A;S19A	Enhanced memory retention	Enhanced memory retention
T18A	Enhanced memory retention	Intermediate
S19A	Enhanced memory retention	Enhanced memory retention
S34A	Enhanced memory retention	Enhanced memory retention
T18D	Enhanced memory retention	Wild-type
S19D	Enhanced memory retention	Wild-type
S34D	Wild-type	Wild-type

Table 2. Summary of short-term (STAM) and long-term (LTAM) memory phenotypes of the MSI-1 phospho-mutants. Wild-type: no significant (pBonf.>0.05) or slightly significant (0.01<pBonf.<0.05) difference between the memory performance of N2 (WT) and the mutant tested 1 hour (STAM) or 24 hours (LTAM) after conditioning. Enhanced memory retention: significant difference in memory performance between N2 (WT) and the mutant tested. Intermediate: memory retention of the mutant shows considerable significant difference (pBonf.<0.01) from both N2 (WT) and *msi-1(os1)* deletion mutant. Comparison of different memory phenotypes were based on the evaluation of Bonferroni corrected p-values (pBonf.) acquired from *post hoc* t-tests.

4 DISCUSSION

In a previous study we reported that loss of *msi-1* in *C. elegans* leads to enhanced memory retention (Hadziselimovic et al., 2014), however, the underlying regulatory mechanisms controlling MSI-1 activity were unknown. Here, we investigated changes of MSI-1 protein abundance and effect of post-translational modifications on the activity of MSI-1 during memory.

First, we tested the MSI-1 protein levels and could not detect any changes in total MSI-1 protein abundance during learning and memory (Fig 1A, B), suggesting that MSI-1 activity might be modulated by post-translational modifications rather than at the protein level. However, we cannot fully rule out subtle or localized protein level changes during memory.

In accord with the possible role of post-translational modifications, we showed that *C. elegans* MSI-1 protein is phosphorylated at residues T18, S19 and S34 at the N-terminal end of the protein and that this phosphorylation plays a crucial role in the protein's function. Furthermore, we have shown that RNA-binding could affect the MSI-1 phosphorylation status, since the phosphorylation of the RNA-binding mutant was impaired (Fig 1C). Thus, our results suggest that the interaction of MSI-1 with its target RNA is necessary for the protein phosphorylation and RNA plays an important role for the assembly and generation of an active MSI-1 protein complex. In line with this hypothesis, links between RNA-binding and protein phosphorylation have been established (Kisielnicka, Minasaki, & Eckmann, 2018; Navarro, Chakravarty, & Nurse, 2017; Spike, Huelgas-Morales, Tsukamoto, & Greenstein, 2018). This suggests that phosphorylation may, indeed, affect the interaction of MSI-1 with its RNA targets.

Interestingly, interference of MSI-1 phosphorylation through individual or simultaneous T/S to A mutations coherently phenocopied the *msi-1* loss-of-function phenotype for both short- and long-term memory (Figs 3A, B and 4A, B). Thus, our results show that phosphorylation at these

threonine and serine residues is essential for MSI-1 activity. The effects of the phospho-inhibitory mutations are not additive, since the memory phenotype of the simultaneous T/S to A mutations is comparable to that of the single mutations (Figs 3A, B and 4A).

Based on our mass spectrometry analysis it was not possible to discriminate whether T18 or S19 is the phosphorylated residue in ETSPPVDGHEEAR trypsin-digested MSI-1 peptide (Fig 2C). However, our memory phenotyping assays indicate that phosphorylation of S19 rather than T18 is important for MSI-1 activity. Consistent with this hypothesis, *msi-1(T18A)* shows intermediate LTAM, while *msi-1(T18A;S19A)* double phospho-mutant has an impaired LTAM similar to the single *msi-1(S19A)* form (Fig 4A, B). Moreover, the substitution of T18 with aspartic acid does not fully emulate phosphorylation (Fig 4C), which might be due to the fact that T18 modifications abrogate *msi-1* function irrespective of phosphorylation. In addition, we observed differences in short- versus long-term behavior of *msi-1(T18D)* and *msi-1(S19D)* (Figs 3C, D and 4C, D), which could be attributed to distinct underlying molecular characteristics, raising the possibility that phosphorylation-dependent MSI-1 promoted memory loss does not equally contribute to short- and long-term memory extinction. This is in line with previous reports suggesting a distinct nature of the molecular mechanisms underlying short- and long-term memory processing (Kauffman, Ashraf, Corces-Zimmerman, Landis, & Murphy, 2010; Tully, Preat, Boynton, & Del Vecchio, 1994).

Strikingly, we found that both T/S to A or T/S to D substitutions of T18 and S19 sites confer enhanced short-term memory retention (Fig 3B-D). The observed identical STAM phenotypes of phospho-inhibitory and phospho-mimetic mutations might be explained by the fact that aspartic acid fails to fully mimic phospho-serine or phospho-threonine in their negative charge, size, or geometry. Consequently, aspartic acid substitutions may not be able to entirely capture the effects

of phosphorylation, which results in a loss-of-function phenotype as it has been reported elsewhere (Chen & Cole, 2015).

In our study we detected a second, less abundant (S34) phosphorylation site (Fig 2D, F). The S34A substitution in MSI-1 protein consistently results in both short- and long-term enhanced memory retention (Figs 3A and 4A), indicating the essential role of phosphorylation at this site regardless of how consolidated the memories are. Furthermore, *msi-1(S34D)* displayed a wild-type phenotype for both short- and long-term memory (Figs 3E and 4E). Therefore, phosphorylation at S34 therefore likely plays a permissive instead of a regulatory role, since MSI-1 with the phospho-mimetic mutations can function similar to the wild-type MSI-1. This is further supported by mass spectrometry data that shows no changes in relative phosphorylation of LNADSDDGSHGSQDPGK peptide upon learning and memory (Fig 2F), thus reflecting a constant phosphorylation state of S34 site.

Altogether, our study highlights the critical role of post-translational modifications of MSI-1 in *C. elegans* and demonstrates that phosphorylation is essential for the activity of MSI-1 during forgetting.

5 REFERENCES

Arribere, J. A., Bell, R. T., Fu, B. X., Artiles, K. L., Hartman, P. S., & Fire, A. Z. (2014). Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in Caenorhabditis elegans. *Genetics, 198*(3), 837-846. doi:10.1534/genetics.114.169730

Arumugam, K., Macnicol, M. C., & Macnicol, A. M. (2012). Autoregulation of Musashi1 mRNA translation during Xenopus oocyte maturation. *Mol Reprod Dev, 79*(8), 553-563. doi:10.1002/mrd.22060

Arumugam, K., MacNicol, M. C., Wang, Y., Cragle, C. E., Tackett, A. J., Hardy, L. L., & MacNicol, A. M. (2012). Ringo/cyclin-dependent kinase and mitogen-activated protein kinase signaling pathways regulate the activity of the cell fate determinant Musashi to promote cell cycle re-entry in Xenopus oocytes. *J Biol Chem, 287*(13), 10639-10649. doi:10.1074/jbc.M111.300681

Awasthi, A., Ramachandran, B., Ahmed, S., Benito, E., Shinoda, Y., Nitzan, N., . . . Dean, C. (2019). Synaptotagmin-3 drives AMPA receptor endocytosis, depression of synapse strength, and forgetting. *Science, 363*(6422). doi:10.1126/science.aav1483

Bargmann, C. I., Hartwieg, E., & Horvitz, H. R. (1993). Odorant-selective genes and neurons mediate olfaction in C. elegans. *Cell, 74*(3), 515-527. doi:10.1016/0092-8674(93)80053-h

Belew, M. S., Bhatia, S., Keyvani Chahi, A., Rentas, S., Draper, J. S., & Hope, K. J. (2018). PLAG1 and USF2 Co-regulate Expression of Musashi-2 in Human Hematopoietic Stem and Progenitor Cells. *Stem Cell Reports, 10*(4), 1384-1397. doi:10.1016/j.stemcr.2018.03.006

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics, 77*(1), 71-94.

Chen, Z., & Cole, P. A. (2015). Synthetic approaches to protein phosphorylation. *Curr Opin Chem Biol, 28*, 115-122. doi:10.1016/j.cbpa.2015.07.001

Choi, Y. M., Kim, K. B., Lee, J. H., Chun, Y. K., An, I. S., An, S., & Bae, S. (2017). DBC2/RhoBTB2 functions as a tumor suppressor protein via Musashi-2 ubiquitination in breast cancer. *Oncogene, 36*(20), 2802-2812. doi:10.1038/onc.2016.441

de Quervain, D., Schwabe, L., & Roozendaal, B. (2017). Stress, glucocorticoids and memory: implications for treating fear-related disorders. *Nat Rev Neurosci, 18*(1), 7-19. doi:10.1038/nrn.2016.155

Gao, Y., Shuai, Y., Zhang, X., Peng, Y., Wang, L., He, J., . . . Li, Q. (2019). Genetic dissection of active forgetting in labile and consolidated memories in Drosophila. *Proc Natl Acad Sci U S A, 116*(42), 21191-21197. doi:10.1073/pnas.1903763116

Hadziselimovic, N., Vukojevic, V., Peter, F., Milnik, A., Fastenrath, M., Fenyves, B. G., . . . Stetak, A. (2014). Forgetting is regulated via Musashi-mediated translational control of the Arp2/3 complex. *Cell, 156*(6), 1153-1166. doi:10.1016/j.cell.2014.01.054

Hentze, M. W., Castello, A., Schwarzl, T., & Preiss, T. (2018). A brave new world of RNA-binding proteins. *Nat Rev Mol Cell Biol, 19*(5), 327-341. doi:10.1038/nrm.2017.130

Himmelreich, S., Masuho, I., Berry, J. A., MacMullen, C., Skamangas, N. K., Martemyanov, K. A., & Davis, R. L. (2017). Dopamine Receptor DAMB Signals via Gq to Mediate Forgetting in Drosophila. *Cell Rep, 21*(8), 2074-2081. doi:10.1016/j.celrep.2017.10.108

Hyman, S. E. (2008). A glimmer of light for neuropsychiatric disorders. *Nature, 455*(7215), 890-893. doi:10.1038/nature07454

Imai, T., Tokunaga, A., Yoshida, T., Hashimoto, M., Mikoshiba, K., Weinmaster, G., . . . Okano, H. (2001). The neural RNA-binding protein Musashi1 translationally regulates mammalian

numb gene expression by interacting with its mRNA. *Mol Cell Biol, 21*(12), 3888-3900. doi:10.1128/MCB.21.12.3888-3900.2001

Inoue, A., Sawatari, E., Hisamoto, N., Kitazono, T., Teramoto, T., Fujiwara, M., . . . Ishihara, T. (2013). Forgetting in C. elegans is accelerated by neuronal communication via the TIR-1/JNK-1 pathway. *Cell Rep, 3*(3), 808-819. doi:10.1016/j.celrep.2013.02.019

Iwaoka, R., Nagata, T., Tsuda, K., Imai, T., Okano, H., Kobayashi, N., & Katahira, M. (2017). Structural Insight into the Recognition of r(UAG) by Musashi-1 RBD2, and Construction of a Model of Musashi-1 RBD1-2 Bound to the Minimum Target RNA. *Molecules, 22*(7). doi:10.3390/molecules22071207

Kauffman, A. L., Ashraf, J. M., Corces-Zimmerman, M. R., Landis, J. N., & Murphy, C. T. (2010). Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. *PLoS Biol, 8*(5), e1000372. doi:10.1371/journal.pbio.1000372

Kim, H., Ishidate, T., Ghanta, K. S., Seth, M., Conte, D., Jr., Shirayama, M., & Mello, C. C. (2014). A co-CRISPR strategy for efficient genome editing in Caenorhabditis elegans. *Genetics, 197*(4), 1069-1080. doi:10.1534/genetics.114.166389

Kisielnicka, E., Minasaki, R., & Eckmann, C. R. (2018). MAPK signaling couples SCF-mediated degradation of translational regulators to oocyte meiotic progression. *Proc Natl Acad Sci U S A, 115*(12), E2772-E2781. doi:10.1073/pnas.1715439115

Kramar, C. P., Chefer, V. I., Wise, R. A., Medina, J. H., & Barbano, M. F. (2014). Dopamine in the dorsal hippocampus impairs the late consolidation of cocaine-associated memory. *Neuropsychopharmacology, 39*(7), 1645-1653. doi:10.1038/npp.2014.11

Kudinov, A. E., Deneka, A., Nikonova, A. S., Beck, T. N., Ahn, Y. H., Liu, X., . . . Boumber, Y. (2016). Musashi-2 (MSI2) supports TGF-beta signaling and inhibits claudins to promote

non-small cell lung cancer (NSCLC) metastasis. *Proc Natl Acad Sci U S A, 113*(25), 6955-6960. doi:10.1073/pnas.1513616113

Liu, Y., Du, S., Lv, L., Lei, B., Shi, W., Tang, Y., . . . Zhong, Y. (2016). Hippocampal Activation of Rac1 Regulates the Forgetting of Object Recognition Memory. *Curr Biol, 26*(17), 2351-2357. doi:10.1016/j.cub.2016.06.056

MacNicol, A. M., Hardy, L. L., Spencer, H. J., & MacNicol, M. C. (2015). Neural stem and progenitor cell fate transition requires regulation of Musashi1 function. *BMC Dev Biol, 15*, 15. doi:10.1186/s12861-015-0064-y

MacNicol, M. C., Cragle, C. E., McDaniel, F. K., Hardy, L. L., Wang, Y., Arumugam, K., . . . MacNicol, A. M. (2017). Evasion of regulatory phosphorylation by an alternatively spliced isoform of Musashi2. *Sci Rep, 7*(1), 11503. doi:10.1038/s41598-017-11917-3

Mariol, M.-C., Walter, L., Bellemin, S., & Gieseler, K. (2013). A Rapid Protocol for Integrating Extrachromosomal Arrays With High Transmission Rate into the C. elegans Genome. *Journal of Visualized Experiments*(82). doi:10.3791/50773

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251. doi:10.1126/science.287.5451.248

Mello, C. C., Kramer, J. M., Stinchcomb, D., & Ambros, V. (1991). Efficient gene transfer in C.elegans: extrachromosomal maintenance and integration of transforming sequences. *EMBO J, 10*(12), 3959-3970.

Muller-McNicoll, M., Rossbach, O., Hui, J., & Medenbach, J. (2019). Auto-regulatory feedback by RNA-binding proteins. *J Mol Cell Biol, 11*(10), 930-939. doi:10.1093/jmcb/mjz043

Navarro, F. J., Chakravarty, P., & Nurse, P. (2017). Phosphorylation of the RNA-binding protein Zfs1 modulates sexual differentiation in fission yeast. *J Cell Sci, 130*(24), 4144-4154. doi:10.1242/jcs.208066

Nuttley, W. M., Atkinson-Leadbeater, K. P., & Van Der Kooy, D. (2002). Serotonin mediates food-odor associative learning in the nematode Caenorhabditiselegans. *Proc Natl Acad Sci U S A, 99*(19), 12449-12454. doi:10.1073/pnas.192101699

Okano, H., Kawahara, H., Toriya, M., Nakao, K., Shibata, S., & Imai, T. (2005). Function of RNA-binding protein Musashi-1 in stem cells. *Exp Cell Res, 306*(2), 349-356. doi:10.1016/j.yexcr.2005.02.021

Onyike, C. U. (2016). Psychiatric Aspects of Dementia. *Continuum (Minneap Minn), 22*(2 Dementia), 600-614. doi:10.1212/CON.0000000000000302

Sakakibara, S., Nakamura, Y., Yoshida, T., Shibata, S., Koike, M., Takano, H., . . . Okano, H. (2002). RNA-binding protein Musashi family: roles for CNS stem cells and a subpopulation of ependymal cells revealed by targeted disruption and antisense ablation. *Proc Natl Acad Sci U S A, 99*(23), 15194-15199. doi:10.1073/pnas.232087499

Spears, E., & Neufeld, K. L. (2011). Novel double-negative feedback loop between adenomatous polyposis coli and Musashi1 in colon epithelia. *J Biol Chem, 286*(7), 4946-4950. doi:10.1074/jbc.C110.205922

Spike, C. A., Huelgas-Morales, G., Tsukamoto, T., & Greenstein, D. (2018). Multiple Mechanisms Inactivate the LIN-41 RNA-Binding Protein To Ensure a Robust Oocyte-to-Embryo Transition in Caenorhabditis elegans. *Genetics, 210*(3), 1011-1037. doi:10.1534/genetics.118.301421

Sutherland, J. M., Fraser, B. A., Sobinoff, A. P., Pye, V. J., Davidson, T. L., Siddall, N. A., . . . McLaughlin, E. A. (2014). Developmental expression of Musashi-1 and Musashi-2 RNA-binding proteins during spermatogenesis: analysis of the deleterious effects of dysregulated expression. *Biol Reprod, 90*(5), 92. doi:10.1095/biolreprod.113.115261

Tully, T., Preat, T., Boynton, S. C., & Del Vecchio, M. (1994). Genetic dissection of consolidated memory in Drosophila. *Cell, 79*(1), 35-47. doi:10.1016/0092-8674(94)90398-0

Vukojevic, V., Gschwind, L., Vogler, C., Demougin, P., de Quervain, D. J., Papassotiropoulos, A., & Stetak, A. (2012). A role for alpha-adducin (ADD-1) in nematode and human memory. *EMBO J, 31*(6), 1453-1466. doi:10.1038/emboj.2012.14

6 DECLARATION BY CANDIDATE

I declare herewith that I have independently carried out the doctoral thesis with the title 'Phosphorylation of MSI-1 is required for forgetting in *Caenorhabditis elegans*'. Only allowed resources were used and all references used were cited accordingly.

Date: 22.07.2020

Signature: 

7 CURRICULUM VITAE

Csaba Boglari

Education

- Ph.D. at the Department of Molecular Neuroscience at Faculty of Psychology, University of Basel, Switzerland (Papassotiropoulos Group) — 08/2020 (expected)
- M.Sc. in Molecular Genetics Cell & Developmental Science at Eötvös Loránd University (ELTE) of Budapest, Hungary (Vellai Group) — 06/2015
- B.Sc. in Biology at ELTE, Budapest, Hungary — 06/2013

Work experience

- Research assistant at Transfaculty Research Platform Molecular & Cognitive Neurosciences, University of Basel, Switzerland: molecular biology, protein engineering, mass spectrometry, proteomics, gene set enrichment analyses, confocal microscopy — since 10/2015

Collaborations, workshops, scientific visits

- Colorado State University, Fort Collins, CO, USA: investigation of trafficking and membrane dynamics of a glutamatergic ion channel in *C. elegans* neurons — 04/2019
- GENiE Protein-DNA Interaction Workshop, Seville, Spain: seminars on DamID and ChIP-seq experimental design and data analysis — 04/2016
- Roche Molecular Systems Inc., Pleasanton, CA, USA: seminars on next generation sequencing, in vitro diagnostics, HPV genotyping — 07/2014

Computer skills

- Basic programming skills (R, Python)
- Statistical and data visualization softwares (SPSS, GraphPad)
- Microsoft Office Word, Excel, PowerPoint

Language skills

- Hungarian (native)
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- German (intermediate level)

Fields of interest

- Molecular Neuroscience
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- Drug target identification & drug development

Teaching activities

- B.Sc. thesis supervisor: Giorgio Buzzi Di Marco 2019/2020
- B.Sc. thesis supervisor: Din Burazorovic 2018/2019
- Project supervisor of B.Sc. seminar students 2017/2019
- B.Sc. thesis supervisor: Tabea Greter 2016/2017
- M.Sc. thesis co-supervisor: Jan Kissling 2016/2017

Conferences

- Boglari, C., Arnold, A., Peter, F., Mastrandreas, P., Papassotiropoulos, A., de Quervain, D. J.-F., Stetak, A. (2018) *Phosphorylation of MSI-1 modulates its function during forgetting in C. elegans*. Poster presented at the CeNeuro 2018 Meeting, Madison, Wisconsin, USA, from 25th to 28th June, 2018

- Boglari, C., Arnold, A., Peter, F., Papassotiropoulos, A., de Quervain, D. J.-F., Stetak, A. (2017) *Regulation of forgetting via modulating MSI-1 protein activity*. Poster presented at 21st International *C. elegans* Conference, Los Angeles, California, USA, from 21st to 25th June, 2017

- Boglari, C., Lengyel, K., Kovács, R., Dudás, B., Kosztelnik, M., Vellai, T. (2015) *The role of Hox genes in Notch receptor expression in C. elegans.* Poster presented at Hungarian Molecular Life Sciences Conference, Eger, Hungary from 27th to 29th March, 2015

Publications

- **Boglari, C.**, Arnold, A., Peter, F., Mastrandreas, P., de Quervain, D. J.-F., Papassotiropoulos, A., Stetak, A. (2020) *Phosphorylation of MSI-1 is required for forgetting in Caenorhabditis elegans.* Submitted to PLoS Biology

- Freytag, V., Probst, S., Hadziselimovic, N., **Boglari, C.**, Hauser, Y., Peter, F., Gabor Fenyves, B., Milnik, A., Demougin, P., Vukojevic, V., de Quervain, D. J.-F., Papassotiropoulos, A., Stetak, A. (2017) *Genome-Wide Temporal Expression Profiling in Caenorhabditis elegans Identifies a Core Gene Set Related to Long-Term Memory.* J Neurosci 37(28): 6661-6672
- Mastrandreas, P., Vukojevic, V., Peter, F., Arnold, A., **Boglari, C.**, de Quervain, D. J.-F., Papassotiropoulos, A., Stetak, A. (2020) *Pharmacological inhibition of Musashi-mediated forgetting improves memory and age-dependent memory decline in Caenorhabditis elegans.* Under submission to eLife