EXTENDED EXPERIMENTAL PROCEDURES

Materials:

N-acetylimidazole was purchased from Sigma-Aldrich (USA). An expression vector (pGEX2T) for recombinant GST was purchased from GE life science (USA). The parental vector for expression of the triple fusion protein of MBP:PTB:hnRNPA2 LC domain was provided by Dr. Micheal Rosen of Universtiy of Texas Southwestern Medical School.

Expression and Purification of Fusion Proteins:

His_6:GFP or His_6:mCherry linked to the LC domain of hnRNPA2 (residues 181-341) was over-expressed in *E. coli* strain BL21(DE3) and purified with Ni-NTA resin (Qiagen, USA) as described previously (Kato *et al*., 2012). Tyrosine-to-serine mutants of GFP:hnRNPA2 LC were purified in the presence of 2M guanidine hydrochloride. The purified proteins were stored at -20˚C with 50% glycerol. Human PPIA full-length cDNA was cloned into multi-cloning site 2 of pACYC-Duet1 for co-expression with mCherry:hnRNPA2 expression vectors.

Hydrogel-binding Assays:

Hydrogel droplets of mCherry:LC domain of hnRNPA2 were prepared as described (Kato *et al*., 2012). Glycerol stocks of the purified GFP fusion test proteins were diluted to 1μM in 1mL of gelation buffer. The GFP test solution was pipetted into the hydrogel dish so as to soak the hydrogel droplets in the GFP solution. After overnight incubation at 4˚C, a horizontal section of the hydrogel droplet was scanned at both the mCherry and GFP excitation wavelengths on a confocal microscope. GFP signals at a boundary area of the hydrogel droplets were scanned by the program ImageJ (Schneider *et al*., 2012).

Heavy Protein Preparation:

To construct an expression vector for His_6:hnRNPA2 LC domain, PCR fragments of hnRNPA2 LC domain (residues 181-341) were inserted to the BamHI/XhoI site of pHis-parallel vector, and the resulting vector was transformed into *E. coli* BL21(DE3) cells. To prepare the stable-isotope labeled (heavy) hnRNPA2 LC, we followed published procedures (Baxa *et al*., 2007). Briefly, transformed cells were first cultured in 20mL of LB medium overnight. The pre-culture was then inoculated in 500mL of M9 minimum media supplemented with all 20 amino acids (no isotope). The culture was shaken at 37°C until OD_{600} reached 0.6. The cells were harvested and washed twice with cold PBS, and inoculated into 500ml of pre-warmed M9 media supplemented with ring $^{13}C_6$-tyrosine (labeled with ^{13}C on the six carbons of the phenyl ring) and all other 19 amino acids. The culture was shaken at 37˚C for 30 minutes, and protein expression was induced with 1mM IPTG for 3 hours before harvest. The cells were then lysed in denaturing wash buffer (25mM Tris pH 8.0, 15mM imidazole, 5mM BME, 6M guanidine HCl). The protein was purified on Ni-NTA resin and eluted in denaturing wash buffer with 300mM imidazole.

For heavy GST protein, pGEX2T vector was transformed to BL21 cells, and the bacteria were grown and induced in the same manner as described for the heavy His_6-hnRNP A2 LCS. The protein was then purified in its native conformation with glutathione resin (GE Health Sciences).

Acetylation of Recombinant Proteins:

N-Acetyltimidazole was prepared as 2M stock solution in DMSO (Sigma-Aldrich, over molecular sieve), protected under argon gas, and stored at -20˚C. To acetylate denatured (heavy) protein, the protein buffer was exchanged to denaturing reaction buffer (50mM HEPES pH 7.4, 5M GuSCN). The protein was first heated at 95˚C for 5 minutes. After cooling to room temperature (RT), the heavy protein was diluted to 1mg/ml, and acetylated with 30mM NAI at RT for 15 minutes. The reaction was quenched with 0.8M Tris-HCl (pH 8.8) at RT for 15 minutes. The native (light) protein (mCherry:hnRNP A2 fibers or GST, 1mg/ml) was acetylated in native reaction buffer (50mM HEPES pH 7.4, 150mM NaCl) with 30mM NAI at RT for 15 minutes, and quenched as described above. The native and denatured proteins were mixed at a 1:1 ratio, and the buffer was exchanged to the denaturing reaction buffer. The proteins were heated at 95˚C for 5 minutes to be fully denatured.

Mass Spectrometry Analysis and Data Processing:

The buffer of heavy and light protein mixture was exchanged to urea buffer (20mM HEPES pH 7.4, 8M urea, 5 mM BME), and diluted four fold with digestion buffer (100mM Tris pH 8.0, 10mM $CaCl_2$). Chymotrypsin (Roche) was added to a final protease:protein ratio of 1:50 (w/w), and the digestion reaction was performed overnight at RT.

Digested peptides were desalted using SepPak C18 columns (Waters) according to manufacturer's instructions. The SILAC sample was analyzed by LC-MS/MS experiments on an LTQ Velos Pro Orbitrap mass spectrometer (Thermo, San Jose, CA) using the top twenty CID (collision-induced dissociation) method (Olsen *et al*., 2009). MS/MS spectra were searched against a composite database of the human IPI protein database (Version 3.60) and its reversed complement using the Sequest algorithm.

Search parameters allowed for chymotryptic peptides with a static modification of 57.02146 Da on Cys, and a dynamic modification of acetylation (42.01056 Da) on Tyr/Ser/Thr/Lys/Arg/Gln/Asn, oxidation (15.99491 Da) on Met, stable isotope (6.02013 Da) on Tyr, respectively. Search results were filtered to include <1% matches to the reverse data base by the linear discriminator function using parameters including Xcorr, dCN, missed cleavage, charge state (exclude 1+ peptides), mass accuracy, peptide length and fraction of ions matched to MS/MS spectra (Huttlin *et al*., 2010).

The abundances of acetylated peptides in the native and denatured samples were estimated by maximal intensities of the monoisotopic peaks from light and heavy labeled peptides, respectively. To calculate the NAI footprint, the ratio of the heavy and light peptides was used, and normalized by the heavy-to-light ratio of non-acetylated parental peptide.

Calculating Solvent Accessible Surface:

Solvent accessible surface of protein side chains was calculated using the program "areaimol" of CCP4 (Winn *et al*., 2011). For correlation analysis, the atoms forming covalent bonds with the acetyl groups (side chain nitrogen atoms on Lys, Arg or Asn, and side chain oxygen atoms of Tyr, Ser or Thr) were used.

293T Cell Culture and Nuclei Preparation:

The tyrosine-negative DMEM high glucose was prepared according to Dulbecco and Freeman's recipe, except that tyrosine was excluded (Dulbecco and Freeman, 1959). To make light and heavy medium, 100mg of light tyrosine or [$^{13}C_6$] tyrosine was added to the tyrosine negative medium, respectively. These media were further supplemented with 10% dialyzed FBS (Sigma-Aldrich, USA). For heavy tyrosine labeling, 293T cells were cultured in the heavy medium for eight generations with 1:5 splitting for each generation, and tested for heavy amino acids incorporation.

Intact nuclei were purified from 10^8 heavy or light cells, respectively. The cells were harvested by trypsin digestion, and washed twice with ice-cold hypotonic buffer (10mM HEPES pH 7.4, 10mM KCl, 2mM $MgCl_2$, 5mM BME with protease inhibitors). The cells were then incubated on ice for 30 minutes, then grinded on ice with 25 strokes using a tissue homogenizer. Nuclei were collected by spinning at 200x g at 4 ˚C for 10 minutes, and washed with the hypotonic buffer without BME.

Acetylation of 293T Nuclei:

To prepare the native nuclei sample, intact nuclei from light cells were resuspended in 1 ml nuclei buffer (50mM HEPES pH 7.4, 150mM NaCl, 2mM $MgCl_2$ and protease inhibitor). For the denatured nuclei sample, the heavy nuclei were resuspended with 1ml denaturing buffer (50mM HEPES pH 7.4, 5M GuSCN and protease inhibitor), sonicated and heated at 95˚C for 5 minutes. Both heavy and light samples were acetylated with 30mM NAI at RT for 15 minutes, quenched by 0.8M Tris pH 8.8, and mixed together. The mixture was then sonicated, concentrated in denaturing buffer, and heated at 95˚C for 5 minutes.

To prepare samples for NAI footprinting of PARP1, the sample was concentrated in buffer with 8 M urea, diluted with nuclease buffer (20mM HEPES 7.4, 2mM $MgCl_2$, 1% SDS). 2ul of benzonase (Santa Cruz, USA) was added to the sample, which was concentrated 10-fold and diluted to the original volume with nuclease buffer. This procedure was repeated three times to remove DNA (Zhang *et al*., 2013). The sample was digested by chymotrypsin as described above.

To prepare samples for analyzing nuclear hnRNPA2, the sample heated in GuSCN was filtered through 100 kDa cutoff Amicon Ultra. The flow-through was concentrated by 30 kDa Amicon Ultra and digested by chymotrypsin as described above.

2D-HPLC mass spectrometry:

Digested peptides from the nuclear SILAC sample were fractionated using an off-line two dimensional RP-RP-HPLC (basic reverse phase coupled with acidic reverse phase) protocol (Gilar *et al*., 2005). Briefly, lyophilized chymotryptic peptides were resuspended in 500ul buffer A (10mM ammonium formate, pH 10) and injected onto an Agilent 300 Extended C18 column (2.1mm×100mm, 3.5μm particle size). Gradient was developed over 60 minute ranging from 5% to 35% buffer B (10mM ammonium formate, pH 10, 90% acetonitrile) at a flow rate of 0.2ml/min. Seventeen fractions were collected and lyophilized. Peptides were then desalted using SepPak C18 columns and lyophilized. In the second dimension, peptides were separated on a 75um×15cm in-house packed RP column (Maccel 200-3-C18AQ, 3 □m, 200 Å) using a gradient developed over 90 min ranging 0% to 37% buffer B (97% acetonitrile, 0.1% formic

acid) at a flow rate of 300nl/minute. Peptides were directly introduced into the mass spectrometer via a hand-pulled emitter.

Purification of MBP:PTB:hnRNP LC Triple Fusion Protein:

BL21(DE3) cells for co-expression of PPIA and His_6-tagged MBP:PTB:hnRNPA2 LC were cultured in LB medium to OD_{600} of 0.8 at 37˚C and then induced by 0.4mM IPTG at 16˚C overnight. The cells were harvested, resuspended and sonicated in lysis buffer (25mM Tris pH 8.0, 1.5M NaCl, 10mM imidazole, 10% glycerol, 5mM BME). The bacteria lysate was cleared by centrifuging at 18,000 rpm for one hour, and loaded on a Ni-NTA column. The column was then washed with lysis buffer, and His_6-tagged MBP:PTB:hnRNPA2 LC was eluted with 25mM Tris 8.0, 300mM imidazole, 10 % glycerol, 5mM BME. 5nmoles of SNAP-Surface 549 (NEB) was added to the elution, and the protein solution was stirred at 4˚C overnight. The labeled protein was loaded on amylose resin (NEB, USA), and eluted in droplet buffer (25mM HEPES pH 7.4, 100mM NaCl, 10% glycerol) supplemented with 10mM maltose. The protein solution was then centrifuged at 100,000 x g for one hour to remove aggregates.

Liquid-like Droplet Formation by MBP:PTB:hnRNPA2 LC and NAI Footprinting:

Liquid-like droplets were formed by mixing 5uM of MBP:PTB:hnRNPA2 LC with 1uM of RNA (5 copies of UCUCUAAAAA), followed by cleavage of MBP by TEV protease at RT. 30mM NAI was added to the droplet solution at the indicated time points, and the acetylation reaction was quenched 0.8M Tris. The denatured His_6-tagged hnRNPA2 LC (heavy) proteins were acetylated in the modified denaturing reaction buffer (50mM HEPES 7.4, 5M GuSCN, 10% glycerol) with 30mM NAI. The acetylation reaction was quenched 0.8M Tris, and mixed with the native sample. Chymotryptic digestion and mass spec analyses were performed as described above.

GFP:hnRNP A2 Recruitment by Liquid-like Droplet:

Liquid-like droplets formed from MBP:PTB:hnRNPA2 LC and the synthetic RNA substrate were incubated at RT for one hour. 0.1uM of EGFP linked wild-type or mutant hnRNPA2 LC proteins were added to the liquid-like droplet solution, and incubated on cover slides for 5 minutes at RT. The droplets were imaged by DeltaVision Elite fluorescent microscope using a 100x oil lens. EGFP signals inside and outside of the liquid-like droplets were measured by the program Image J. The partition ratio of EGFP:hnRNPA2 protein was calculated by dividing the signal inside the droplet by the signal outside. This ratio was then normalized with the signal intensity of Alexa 549 that had been SNAP tagged onto the PTB:hnRNPA2 LC protein in the droplet.

Localization of PPIA to Stress Granules:

U2OS cells were cultured on chamber slides overnight after seeding. Stress granules were induced by heat shock at 44˚C for 45 minutes. Cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized in PBST (PBS plus 0.2% Triton) for 20 minutes. After blocking with PBTA (3% BSA, 0.2% Triton in PBS) for 30 minutes, cells were incubated with 1:300 goat α-TIA1 (Santa Cruz, USA) and 1:300 rabbit α-PPIA (Abcam, USA) in PBTA overnight at 4˚C. Primary antibodies were developed with 1:300

AlexaFluor 568 donkey α-goat (Invitrogen, USA) and 1:500 AlexaFluor 488 donkey α–rabbit (Invitrogen, USA) for 1 hour at RT. After washing with PBST, the cells were mounted with VECTASHIELD mounting medium with DAPI (Vector laboratories, USA), and imaged using a DeltaVision Elite microscope.

Transmission Electron Microscopy of mCherry:hnRNPA2 Fibers:

The recombinant mCherry:hnRNPA2 hydrogel was resuspended to a final concentration of 0.1 mg/ml by brief sonication in wash buffer (25 mM HEPES pH 7.4, 150 mM NaCl). Freshly glow-discharged (40 mA, 90 seconds) carbon-coated copper grids (Electron Microscopy Sciences, USA) were floated on the fiber sample solution for 2 minutes for adsorption. The grids were washed in the wash buffer 3 times, and then stained with 2% sodium phosphotungstate (pH 7.0) for 1 minute. TEM images were taken on a Tecnai Spirit microscope at 67,000x magnification.

Supplemental Table S1. Footprint Deduced by NAI-mediated acetylation of GST. Related to Figure 1.

Residue Number	Log_2(Denatured/Native)	Solvent Accessible Surface ($Å^2$)
S2	0.94	0
Y7	2.07	9.1
Y23	0.25	35.3
K27	0.58	14.7
Y28	1.69	0.5
Y33	1.48	0.2
K40	-0.74	53.5
Y57	2.82	0.3
Y58	3.45	0.1
K64	-0.58	32.1
Y104	1.06	7
Y111	0.34	32.1
N143	2.20	0
T149	1.99	0
Y156	1.99	8.8
K180	0.70	23.8
K181	1.19	18
R182	-0.09	23.1
Y192	2.37	0.7

Supplemental Table S2. Footprint Deduced by NAI-mediated acetylation of PARP. Related to Figure S1.

Residue Number	Log_2 (Denatured/Native)	Solvent Accessible Surface ($Å^2$)
K571	0.74	20.8
K616	1.54	2.9
K621	0.15	31.8
S782	2.3	0
S783	1.4	0.2
T799	1.5	39.8
K802	0.7	20.8
S808	0.65	50
K816	1.2	0
Y817	0.1	44.5
S902	0.18	52.8
K903	1.38	7.1
S904	0.55	42
Y907	0.55	36.9

Supplemental Table S3. Footprint Deduced by NAI-mediated acetylation of hnRNPA2. Related to Figure 2, 3, 5.

Residue Number	mCherry:hnRNPA2 Hydrogel	Nuclei	PPIA WT	PPIA R55A	MBP:PTB:LC no TEV	PTB:LC Droplet 10 min	PTB:LC Droplet 2 hours	PTB:LC Droplet 18 hours
S219	-0.26	-0.11	-0.05	0.09	0.13	0.22	0.19	0.21
Y222	0.34	0.09	0.55	0.59	0.25	-0.12	0.44	0.13
S224	-0.25							
Y232	0.34	0.21	0.27	0.14	0.05	0.25	0.15	0.28
N243	0.28							
S247	-1.03	-0.15	-0.29	-0.13	0.32	-0.03	0.19	-0.08
Y250	0.19	0.01	0.26	0.28	-0.13	0.28	0.22	0.45
Y257	-0.02							
N282	1.77							
Y283	1.59	0.46	1.52	1.52	0.35	1.08	1.25	1.63
S285	2.18	0.50	2.30	2.56	0.65	1.52	1.89	2.32
Y288	1.50	0.61	1.79	1.77	0.46	1.03	1.58	1.66
Y294	0.98	0.33	1.56	1.63	0.48	0.84	1.23	1.32
S299	2.92	0.32	2.82	2.63	0.74	1.05	2.68	2.54
Y301	2.28	0.51	2.10	2.00	0.32	1.08	1.88	2.31
K305	-0.10	0.02	-0.03	-0.01	0.1	-0.05	-0.14	0.08
S306	2.69							
S312	1.66	0.49	1.54	1.63	0.43	1.02	1.2	1.72
Y319	2.04	0.57	1.80	1.65	0.65	1.33	1.58	1.65
Y324	1.74	-0.16	-0.26	1.74	-0.18	-0.28	0.13	0.05
S329	0.14	-0.04	0.03	0.04	0.08	0.14	0.08	0.17
S332	-0.25	-0.04	-0.01	0.02	0.05	-0.07	-0.03	-0.18
Y335	-0.03	-0.07	0.03	0.02	-0.18	-0.12	0.19	0.2

Supplementary References

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