

Modulation of the cytoplasmic functions of mammalian post-transcriptional regulatory proteins by methylation and acetylation: a key layer of regulation waiting to be uncovered?

Tajekesa K.P. Blee*, Nicola K. Gray*¹ and Matthew Brook*

*MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland U.K.

Abstract

Post-transcriptional control of gene expression is critical for normal cellular function and viability and many of the proteins that mediate post-transcriptional control are themselves subject to regulation by post-translational modification (PTM), e.g. phosphorylation. However, proteome-wide studies are revealing new complexities in the PTM status of mammalian proteins, in particular large numbers of novel methylated and acetylated residues are being identified. Here we review studied examples of methylation/acetylation-dependent regulation of post-transcriptional regulatory protein (PTRP) function and present collated PTM data that points to the huge potential for regulation of mRNA fate by these PTMs.

Introduction

Tight regulation of gene expression is central to normal cell function; for example, in co-ordinating cellular growth and proliferation, differentiation and response to stress. This regulation occurs at multiple levels; including transcription and mRNA processing in the nucleus, mRNA export, mRNA translation and stability in the cytoplasm and post-translational modification (PTM) of gene products. Recent findings have emphasized the importance of post-transcriptional control mechanisms, which are mediated by numerous, often multifunctional, proteins including mRNA-binding proteins (RBPs). RBPs are involved in each step of mRNA metabolism, binding their cognate mRNAs [1] to form a messenger ribonucleoprotein (mRNP) complexes and acting either directly (e.g. deadenylases) or indirectly, via recruitment of other factors, to determine mRNA fate. RBP–RNA interaction can begin during transcription and be sustained until the RNA is degraded or occur transiently to mediate or regulate specific processes, e.g. splicing, transport, localization, translation and decay [2]. In this regard, some RBPs function as chaperones to help RNA fold into

higher order structures which, together with specific RNA sequences, can recruit additional/alternative RBPs [3,4].

Many RBPs and mRNP-associated proteins [here termed post-transcriptional regulatory proteins (PTRPs) for brevity] are themselves subject to extensive control e.g. by PTM, of which phosphorylation is the best-characterized example. Previously, however, non-phosphorylation PTMs with equally important regulatory functions have emerged, producing a growing library of reversibly and differentially-modified cytoplasmic PTRPs, including RBPs, whose modification state may alter their structure/conformation and thereby regulate their function, e.g. by influencing their cellular localization, RNA-binding activity, protein–protein interactions or combinations thereof [5]. Using selected examples of mammalian cytoplasmic PTRPs we summarize the functional roles of arginine methylation, the most studied non-phosphorylation PTM in this regard. Emerging roles of non-histone lysine acetylation (acK) and methylation (meK) and the putative utilization of differential modifications at single lysine residues as switches for controlling mRNA fate will also be discussed. Finally, we will present collated data from multiple published high- (HT) and low-throughput (LT) proteomic studies (Table 1) that point to the potential untapped regulatory consequences of such PTMs in the cytoplasmic co-ordination of mRNA utilization.

Key words: arginine methylation, lysine acetylation, lysine methylation, messenger ribonucleic acid (mRNA) translation, messenger ribonucleic acid (mRNA) turnover, post-transcriptional control, post-translational modification, ribonucleic acid (RNA)-binding proteins.

Abbreviations: acK, lysine acetylation; AdOx, adenosine dialdehyde; ALS, amyotrophic lateral sclerosis; ARE, AU-rich elements; DDX4, dead-box protein 4; eEF1A, eukaryotic elongation factor 1- α ; eIF5A, eukaryotic translation initiation factor 5A; ELAVL1, ELAV-like protein; FMRP, fragile X mental retardation protein; FTL, frontotemporal lobar degeneration; HDAC, histone deacetylase; hyp, hypusination; KAT, lysine acetyltransferase; KDAC, lysine deacetylase; KDMT, lysine demethylase; KMT, lysine methyltransferase; MBT, malignant brain tumour; meK, methyllysine; meR, methylarginine; mRNP, messenger ribonucleoprotein; PABP1, poly(A)-binding protein 1; PRMT, protein arginine methyltransferase; PTM, post-translational modification; PTRP, post-transcriptional regulatory protein; RBPs, mRNA binding proteins; RGG, arginine-glycine-glycine; TDP-43, transactive response DNA-binding protein 43 kDa.

¹ To whom correspondence should be addressed (email nicola.gray@ed.ac.uk).

Table 1 | Collated high-confidence post-transcriptional effector protein acKs and/or methylations from mass spectrometric studies

acK residues detected in at least two mammalian proteomic studies are shown whereas, due to the low numbers of studies available, all meK residues in proteins of interest are shown; studies are referenced for each numbered residue. Protein name and amino acid numbering refer to the 'canonical' human form of a protein, as annotated in UniProt, unless otherwise stated. Where multiple protein isoforms are named all PTMs apply to all isoforms unless otherwise stated. Where a PTM occurs on a residue in a non-canonical protein isoform the isoform designation is shown in square brackets alongside the residue number (e.g. 1 [1]). § eIF4G1 residue 190 can also correspond to residue 150 [B]/197 [9]; †KHSTAP1 Lys¹⁹⁴ is not conserved from mouse to human. CNBP, cellular nucleic acid binding protein; DAZAP1, DAZ-associated protein 1; DDX1, DEAD-box protein 1; eEF1A1, eukaryotic translation elongation factor 1A1; eIF, eukaryotic initiation factor; eRF3A, eukaryotic translation termination release factor 3A; EWSR1, RNA-binding protein EWS; FUS, RNA-binding protein FUS; hnRNP, heterogeneous nuclear ribonucleoprotein; KSRP, KH type-splicing regulatory protein; PABP1, poly(A)-binding protein 1; PAIP2, PABP-interacting protein 2; PARN, poly(A)-specific ribonuclease; PTB1, polypyrimidine tract-binding protein; SERBP1, plasminogen activator inhibitor 1 RNA-binding protein; TAF15, TATA-binding protein-associated factor 15/RNA-binding protein 56.

Protein	acK residue	meK residue	Refs	Protein	acK residue	meK residue	Refs	Protein	acK residue	meK residue	Refs		
CNBP	8		[38,80,81]	eEF1A1 (cont'd)	395		[31,38,80,82,83]	eIF4B (cont'd)	557		[83,84]		
	103		[80,81]		408	408	Ac:[38,83]/Me: [67]		586			[31,84]	
DAZAP1	59		[38,83]		439		[31,38,80,82,83,85,87-89]		592			[37,38]	
	103		[31,82]		443		[31,83,87]	eIF4E	119			[37,83-85]	
	150		[90]	eIF1b	109		[37,83]		eIF4G1	190 [A + 8] [§]			[37,38,80,84,85]
DDX1	117		[80,91]		eIF2α	141		[31,37,38,80,83-85]		597			[83,85]
	239		[31,37,38,85]	eIF2Bα			145	[86]		950			[37,80,88]
	268		[31,83]		eIF3A		2[2]	[86]	eIF4H	80			[80,82,84]
	281		[31,37,38,80,83,85]	68			[31,37,38,80,82-85]	123				[38,84]	
	596		[37,38,84,85]	538			[37,38]	eIF5A-1		214		130	[86]
eEF1A1	41	36	[69,92]	eIF4A1		54				[37,82-84]	245		
	44		[38,80,82,83,85,88]		68		[38,83]		27 [1]			[31,84]	
		51	[92]		118		[31,37,38,83,85]	47 [1]			[31,37,83,84,93]		
	55	55	Ac: [82]/Me: [67,92]		146		[37,38,80,83,85]	49 [1] or 69 [2]			[38,80,82,85]		
	62		[92]	174		[31,37,80,82,84,85]	77[2]			[31,37,38,80,82,85,88]			
	79	79	Ac: [87]/Me: [67,92]	193		[37,38,80,82-85,91]	97[2]			[38,80,82]			
	84	84	Ac: [31,87,88]/Me: [67,92]	238		[38,80,83-85]	151 [2]			[37,38,80,85]			
	146		[38,80,82,83,85,88]	eIF4B	291		[31,37,38,84]	eRF3A	345 [2]			[38,80]	
	154		[38,67,83]		309		[31,37,38,80,82,84,85]		377 [2]			[37,38]	
	165	165	Ac: [38,82,83,85,89]/ Me: [67,69,92]		381		[37,38,80,85]		391 [2]			[82,83]	
	172	172	Ac:[31,38,80,82,83,85,88,89,91]/Me: [69]	eIF4B	177		[37,38]	627 [2]			[37,38]		
	179		[80-83,85,88,89,91,94]		194		[37,38,84]	EWSR1	438 [3]			[37,38,80,84,85]	
	180		[31,82]		223		[37,38]		444 [5]			[37,38,80,82,84,88]	
	212		[38,85]		365		[37,38,83,84]		646 [5]			[82,84]	
	244		[38,83]		395		[84]		649 [5]			[38,82,84]	
	255		[38,80,82,83,85,88]		FUS	327 (mouse)		[81,84]	hnRNP1/2	8			[38,80]
	273		[31,38,80,82,83,85,89]			332		[38,80,82]					
	318	318	Ac: [38,80,82]/Me: [42,67,92]	447 [2]		[37,38,85]							
	392		[31,38,80,82,83,85,89,91,94]										

Table 1 | Continued

Protein	acK residue	meK residue	Refs	Protein	acK residue	meK residue	Refs
hnRNP C1/2 (cont'd)	39		[31,38,80,82,83]	PABP1	78		[38,80,84,85]
		98	[86]		95		[62]
	144 (C1)		[80,84]		104		[31,37,38,80,82-85]
	203 (C1)		[31,38,80]		108		[31,38,80,82,84,88]
	204 (C2)		[83,84]		157		[82,84]
	216		[37,38,82]		188	188	Ac: [62,84,86]/Me: [62]
	219		[80,82]		259		[31,37,38,84]
hnRNP (Isoforms 1 + 3)	72 [1-4]		[80]		299	299	Ac: [37,38,62,80,85]/Me: [62]
	114		[37,38,80,82,83,85]		312	312	Ac: [62]/Me: [62]
	119		[83]		361	361	Ac: [62,83]/Me: [62]
	129		[37,38,80,82]		606	606	Ac: [62]/Me: [62]
	153		[80]		620		[37,38,83]
	165		[31,38,80,85]		PAIP2	123	
	176		[82,84]	PARN	220		[31,38,85]
	197		[37,38,80,83,85]		499		[31,38,80]
	218		[38,85]	PTB1	436		[80,85]
	231		[38,83]	KHSTAP1 (Sam68)	96		[37,38,84,85]
	243		[37,80,82]		165		[37,82]
251		[37,38,82,83]	169			[31,38,80,88]	
			175			[31,37,38,80,81,83-85,88]	
hnRNP-like	76	76	Ac: [82,86]/Me: [68]	194*	194*	Ac: [82]/Me: [66]	
	137		[38,84]		200	[66]	
	161	161	Ac: [80,82]/Me: [66]	SERBP1	68		[31,37,38,80,84]
	204		[37,38,82]		102		[38,84]
	216		[37,38,80,84,88]		122		[31,37,38,80,84,85,88]
ELAVL1	55	[38,81]	140			[31,80,82,84,88]	
			211			[31,37,38,84]	
KSRP		71	[86]	236		[37,38,85]	
	87		[37,83,84]	TAF15	297		[37,88]
	122		[82,84]				
	169		[31,80,84]				
	359		[83,84]				

arginine methyltransferases (PRMTs). Addition of each methyl group (Figure 1) removes a potential hydrogen bond donor, imparts increasing bulkiness and hydrophobicity but, importantly, has no effect on the cationic charge of an arginine residue [8,9]. Methylation can alter conformation affecting RNA–protein binding, protein–protein interactions and sub-cellular localization [6,9] which has implications for many cellular processes and can result in pathogenesis [6,8,10] (Figure 2). Here, we give two examples of the varied effects of arginine methylation on cytoplasmic PTRP activities.

Fragile X mental retardation protein

Fragile X mental retardation protein (FMRP) is a nucleocytoplasmic shuttling protein whose absence is associated with Fragile X syndrome [11]. It binds approximately 4% of foetal brain mRNAs, is detectable within distinct mRNP complexes and on polyribosomes [12] and has roles in mRNA transport, stability and translational activation and repression [11]. The multi-functionality of FMRP is, in part, mediated by differential binding to RNA motifs, e.g. G-quadruplexes [11] or SoSLIP RNA motifs [13], via its RGG box.

One attractive hypothesis is that PTMs control FMRP multi-functionality and indeed it is a substrate for multiple PRMT1-dependent arginine methylations, with Arg^{533/538/543/545} within the RGG box [14] being of particular note: PRMT1-dependent methylation of specific arginine residue pairs, Arg^{533/538} and Arg^{543/545}, differentially modulates *in vitro* binding to specific target mRNAs [12,14]. Further support for a functional role of FMRP arginine methylation comes from the observed reduction in polyribosomal association when FMRP methylation is inhibited, either via substitution of candidate arginine residues in the RGG box or by the use of global methylation inhibitors, e.g. adenosine dialdehyde (AdOx) [12,14]. However, since this effect could result from the mutation of the arginine residues *per se* and/or altered methylation of non-FMRP targets, these data do not directly demonstrate FMRP functional regulation by methylarginine (meR) residues.

Arginine methylation has also been proposed to regulate FMRP protein interactions. AdOx treatment inhibits FMRP heterodimerization with its paralogues fragile X-related protein (FXR) -1P and 2P, but not FMRP homodimerization [12,15,16], although the functional significance of this remains unclear. Furthermore, although AdOx-treatment did not alter FMRP recruitment to arsenite-induced stress granules (SGs) [17], cytoplasmic foci of repressed mRNAs, it increased the numbers of other FMRP-containing cytoplasmic foci, of unknown function [18]. However, the dependence on FMRP arginine methylation for these observations remains undetermined, given the non-specific inhibitory effects of AdOx. Thus, although it is clear that FMRP is a PRMT1 substrate, the specific *in vivo* functional consequences of its arginine methylation remain to be fully elucidated.

ELAVL1/Hu-antigen R

Embryonic lethal abnormal vision (ELAV)-like protein (ELAVL) 1 is a ubiquitously expressed, essential RBP that is a predominantly nuclear regulator of pre-mRNA splicing. It also shuttles to the cytoplasm where it can bind to AU-rich elements (AREs) in 3'-UTRs [19] to stabilize [20], destabilize [21], translationally repress [22] or activate [23] target mRNAs. Moreover, it can bind to 5'-UTRs to repress translation [24] or activate cap-independent translation [25]. Finally, it can also regulate mRNA fate through interplay with miRNAs [26] with these functions not being mutually exclusive and potentially operating in concert.

In cells, ELAVL1 can be dimethylated at Arg²¹⁷ [27], which lies within the hinge region of its nucleocytoplasmic shuttling sequence that binds nuclear export cofactors [28]. Arg²¹⁷ dimethylation is enhanced during liposaccharide-mediated stimulation of macrophage and leukaemia cell lines concomitant to its relocalization to the cytoplasm. However, although PRMT4-dependent Arg²¹⁷ dimethylation is proposed to regulate both export and nuclear re-import of cytoplasmic ELAVL1, no direct role in this process is yet demonstrated. Arg²¹⁷ dimethylation also modulates mRNA stability with unmethylated ELAVL1 stabilizing and Arg²¹⁷ dimethylated ELAVL1 destabilizing methionine adenosyltransferase 2A (MAT2A) mRNA during liver development and malignant transformation [21]. However, its role in RNA-binding appears complex as methylated ELAVL1 is proposed to concomitantly increase and decrease the stability of some mRNAs (e.g. cyclin A and cyclin-dependent kinase inhibitor 2A (CDKN2A) respectively) to inhibit replicative senescence [29]. Surprisingly, a role for arginine methylation in the best studied function of ELAVL1 in promoting mRNA translation and stability (i.e. pro-inflammatory cytokines, such as Tumour necrosis factor (TNF) α) remains to be demonstrated.

These exemplars illustrate that arginine methylation can elicit profound effects. Given the extent of meR residues in PTRPs and ribosomal proteins, the discovery that this PTM is reversible [6,30] (Figure 1) opens up the possibility that post-transcriptional regulation is likely to be a much greater target for dynamic regulation by arginine methylation than currently appreciated.

Lysine acetylation

The bulk of our knowledge pertaining to the regulatory functions of acK and the effector enzymes that add/remove this PTM (Figure 1) is derived from studies of histone function [31,32]. Acetylation neutralizes the positive charge on a basic lysine residue (inducing a net charge change similar to that caused by phosphorylation; Figure 1), affecting protein structure and conformation and consequently protein activity and/or protein–protein/protein–nucleic acid interactions [31]. The human genome encodes >20 lysine acetyltransferases (KATs) and at least 18 lysine deacetylases (KDACs) [33] but, to date, very few *bona fide* cytoplasmic

Figure 1 | Methylation and/or acetylation of arginine and lysine residues

(A) Arginine is (1) methylated [6,8] at one of the terminal guanidino nitrogen atoms (N^G) by type I-III PRMTs to generate monomethylarginine (MMA). (2) Type I PRMTs then further modify the same nitrogen to generate asymmetrically dimethylated (aDMA) arginine, whereas (3) type II PRMTs modify the second nitrogen group to generate symmetrically dimethylated (sDMA) arginine. aDMA/sDMA PTMs were considered irreversible and could only be 'blocked' by (4) deimination to citrulline by peptidyl arginine deiminases (PADs) [95,96] but (5) PAD4 is suggested to also act on MMA to 'reverse' mono- but not dimethylation. Also Jumonji domain-containing protein (JMJD) 6 catalyses (6) the conversion of aDMA, sDMA or MMA to arginine [97,98]. **(B)** KATs (1) acetylate the epsilon nitrogen of lysine residues to create acetyllysine [32]. This is reversed (2) by KDACs. Acetylation/deacetylation enables the reversible control of the positive charge status of lysine. Lysine can exist in three methylated states (mono-, di- and trimethylated) [57,61] catalysed (3) by KMTs. Not all characterized KMTs can catalyse all methylation states. Lysine demethylation (4) is catalysed by KDMTs [57,59]. The lysine-specific demethylase (LSD) KDMTs can only demethylate mono- and dimethyl-lysine residues.

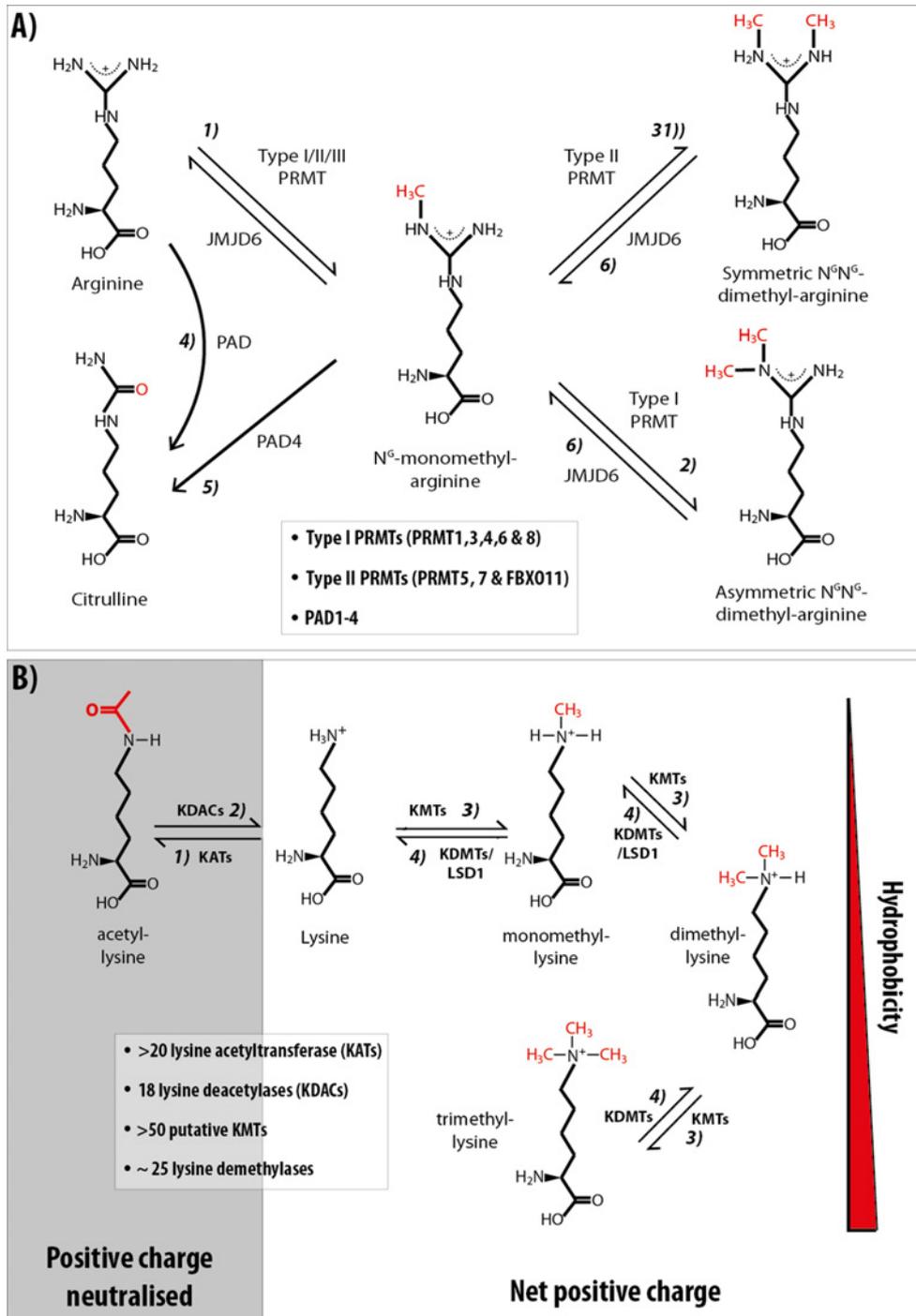
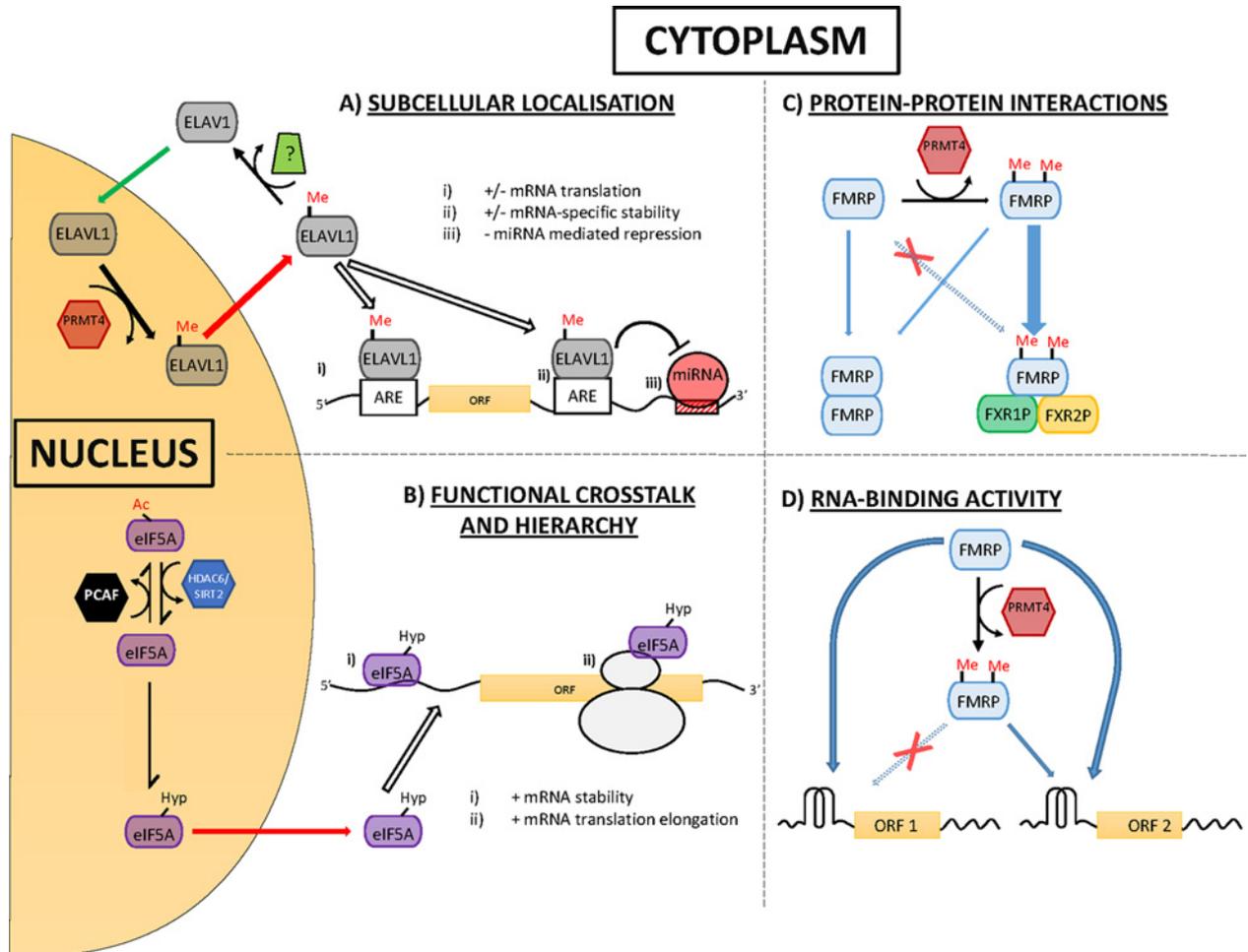


Figure 2 | Functional consequences of PTMs in post-transcriptional regulator proteins

(A) PTM status can alter sub-cellular localization. ELAVL1 is a multi-functional nucleocytoplasmic shuttling RBP whose methylation results in its export from the nucleus, where it participates in splicing, to the cytoplasm where it regulates mRNAs containing AREs in their 5'- or 3'-UTR (i-iii). It is unclear which enzyme removes this PTM (represented by '?') facilitating its nuclear import. (B) PTM at one residue can affect another. eIF5A is hypusinated at Lys⁵⁰ enabling its export to the cytoplasm where it functions in mRNA translation/stability (i and ii). However, Lys⁴⁷ acetylation by PCAF/CBP-associated factor (PCAF) antagonizes h retaining eIF5A in the nucleus. Acetylation may be reversed by HDAC6/Sirtuin (SIRT2) (C) Protein-protein and (D) RNA-protein interactions are PTM sensitive. (C) Non-methylated FMRP binds paralogues FXR1P and FXR2P inefficiently. Methylation by PRMT4 enhances FMRP heterodimerization with FXR1P and FXR2P, but has no apparent effect on FMRP homodimerization, thus regulating the composition of mRNP complexes. (D) PRMT4-directed FMRP methylation at Arg^{533/538} or Arg^{543/545} blocks binding to specific mRNA subsets (ORF 1) but binding to other mRNA targets (ORF 2) is unaffected. Red and green arrows, nuclear export and import respectively; wavy lines mRNA. Blue arrows represent interactions, with broader blue arrows representing enhanced interactions and dotted blue lines with a cross representing impeded interactions.



substrates are identified. Despite this, there has been an explosion in the numbers of putatively identified acKs on non-histone, cytoplasmic proteins [34–36], largely due to improved anti-acK antibody cocktails and ‘stable isotope labelling by amino acids in cell culture’ (SILAC) MS techniques [32,37,38], such that acK is now considered as pervasive as protein phosphorylation. Three examples of PTRPs regulated by acK are described below.

Dead-box polypeptide 4

In mammals, dead-box polypeptide 4 (DDX4) (also called vas-associated (VASA) (human) and mouse VASA homologue (MVH)) is a germline-specific, ATP-dependent RNA helicase which is essential for male fertility [39] and is enriched within the chromatoid body (CB), a granular structure, essential for post-transcriptional regulation during spermatogenesis. Consistent with this localization, DDX4

interacts with several RNA-induced silencing complex (RISC) components [40–42] and is implicated in translational repression mediated by small RNAs (i.e. miRNAs and piRNAs) [42–44]. Lys⁴⁰⁵ acetylation of DDX4 at stages IV–VI (meiotic onset) of spermatogenesis, modulates its binding to a subset of translationally arrested target mRNAs [e.g. eukaryotic translation initiation factor (eIF) 4B] resulting their translational derepression. As eIF4B is a translation factor, this implies the existence of RBP acK-mediated regulatory mechanisms that act as temporal switches for regulating protein synthesis rates. DDX4 is also subject to arginine methylation of unknown function.

eIF5A

eIF5A exists in two isoforms, the ubiquitously expressed eIF5A1 and the less-well characterized testis- and brain-restricted eIF5A2 [45,46]. eIF5A1 undergoes nucleocytoplasmic shuttling and its cytoplasmic functions, studied largely in yeast and non-mammalian vertebrates, include translation elongation and mRNA turnover. Nascent eIF5A1 protein is transported to the nucleus where its hypusination (hyp) at Lys⁵⁰ (hypK⁵⁰) leads to its nuclear export and cytoplasmic retention [47–49]. Although hyp is irreversible, eIF5A1 can also be acetylated at Lys⁴⁷, which antagonizes Lys⁵⁰ hyp and prevents its nuclear export, thereby providing a mechanism to regulate eIF5A1 sub-cellular localization. Furthermore, both Lys⁵⁰ hyp and retention of the positive charge state of Lys⁴⁷ are essential for its translational functions [48,49] and Lys⁴⁷ acetylation therefore provides a fail-safe mechanism to abrogate eIF5A-dependent translation should any non-hypusinated protein escape into the cytoplasm.

This antagonism illustrates the potential for hierarchies and cross-talk between PTMs [48,49]. It is therefore intriguing that eIF5A1 is also apparently subject to multiple additional acKs (Table 1), including on Lys⁴⁹, immediately proximal to the hypusinated Lys⁵⁰, all of which await functional characterization.

Transactive response DNA-binding protein -43

Transactive response (TAR) DNA-binding protein -43 (TDP-43) is a nucleocytoplasmic shuttling protein which is predominantly nuclear localized except in diseased tissue from patients with amyotrophic lateral sclerosis (ALS) and subtypes of frontotemporal lobar degeneration (FTLD), who display cytoplasmic TDP-43-positive aggregates [50–52]. In non-diseased cells, cytoplasmic TDP-43 is involved in mRNA-specific transport into neuronal granules, mRNA-specific turnover and miRNA biogenesis regulation [53]. Lys^{145/192} of the RNA-recognition motif (RRM) of TDP-43 can be acetylated and TDP-43 acetylation mimic mutants (e.g. K–Q) exhibit a 50%–65% decrease in mRNA binding efficiency and readily form cytoplasmic aggregates. Similar wild-type TDP-43 aggregates can also be induced by oxidative stress but co-expression of a histone deacetylase (HDAC6) deacetylates cytoplasmic TDP-43 under basal conditions but not in stress-induced aggregates [51,54]. Thus

TDP-43 acetylation regulates its RNA-binding and sub-cellular localization, although it is unclear whether these two processes are distinct or linked and TDP-43 acK may be dysregulated by environmental stress. Lys¹⁴⁵ acetylation is detected in ALS patient brain/spinal cord inclusions but not those from FTLTDP, in whom TDP-43 is C-terminally truncated and lacks Lys¹⁴⁵ [55]; therefore, it is unclear whether TDP-43 acK is directly involved in the pathogenesis of ALS proteinopathy.

Lysine methylation

Like acetylation, lysine mono-, di- and tri-methylation (Figure 1) are well-established PTMs with profound regulatory functions in chromatin remodelling and transcription [56–58]. To a large extent, the bias toward studying chromatin-associated proteins stems from the relative ease of obtaining large quantities of mammalian nucleosomes and availability of PTM-specific reagents for histones. This contrasts the lack of simple enrichment steps for most mammalian cytoplasmic protein complexes, a lack of reagents for detecting/enriching non-histone meK (methyllysine)-containing proteins and the inefficiency of trypsin digestion at methylated lysine residues which complicates MS analysis. However, ribosomes are highly abundant, overcoming some of these limitations and, consequently, numerous ribosomal subunit proteins are documented as lysine methylated, most often with unknown functional outcomes [57,59].

Whereas lysine acetylation alters net charge, methylation does not and in fact, trimethylation actually stabilizes the positive charge of this side chain (Figure 1). However, incremental methylation adds significant bulk to the lysine residue, having the potential to cause steric effects on protein conformation and/or interactions, whilst simultaneously increasing the hydrophobicity of the residue. Since lysine residues are often surface exposed, contributing to charged surfaces and often involved in RNA–phosphate-backbone interactions [57,60,61], this hydrophobicity change is probably critical in determining protein–nucleic acid as well as protein–protein interactions which underlie post-transcriptional control. However, there are currently few insights into the regulation of PTRPs by lysine methylation. The exceptions are poly(A)-binding protein 1 (PABP1), which contains multiple conserved meK residues [62] and eukaryotic elongation factor 1- α 1 (eEF1A1) which is one of the most repeatedly detected acetylated/methylated proteins in proteomics studies (Table 1), most probably due to its very high cellular concentration, with 21 high-probability acK residues and eight high-probability meK residues (10 in total). eEF1A1 is almost ubiquitously expressed in adult tissues and delivers aminoacylated tRNAs to the ribosome during translation elongation [63]. eEF1A1 also has non-canonical functions in cytoskeletal organization [64] and is implicated in other processes, e.g. protein degradation, receptor recycling, cell proliferation and apoptosis, although the extent to which these reflect genuine, direct non-canonical roles is unclear. Although, the functions of eEF1A1 PTMs are undetermined,

trimethylation of eEF1A1 Lys³¹⁸ was previously documented and is one of several meK residues implicated in chick neural crest migration [65]. Given the conservation of this PTM from yeast (Lys³¹⁶), it is tempting to speculate that eEF1A1 lysine methylation may have similar relevance in mammals.

Although the regulatory capacity of lysine methylation is evident from studies on histone function and supported by the limited evidence reviewed here, the number of verified meK residues on non-histone proteins is very low, with the identification of novel lysine methylation events remaining far from trivial. It seems highly unlikely that the currently documented numbers of meKs is an accurate reflection of this PTM in the proteome, since more than 50 putative lysine methyltransferases (KMTs) and approximately 25 lysine demethylases (KDMTs) are encoded within the human genome, although very few have annotated substrates. Consistent with this view, recent proteomic studies collectively document several hundred novel meK residues (still relatively few compared with the >10 000 acK residues documented) across a broad range of protein classes [66–69] (see Table 1 for examples relating to post-transcriptional control).

Realizing the potential: emerging areas, technological developments and challenges

The discovery of arginine and lysine PTMs has entered something of a ‘boom-time’ due to proteomics using heavy isotope-labelled reagents (e.g. arginine/lysine/methyl donor/acetyl donor), new proteases that efficiently cleave at modified residues and improved antibodies for enriching modified proteins/peptides. Table 1 shows collated high-confidence acK residues (i.e. detected in multiple MS studies) and meK residues identified in PTRPs to date. Although a vast majority of these PTMs are unstudied, collectively, these data, are strongly suggestive of significant unexplored regulation of post-transcriptional control mechanisms. Intriguingly, Table 1 also highlights that the numbers of individual non-histone lysine residues that are subject to differential acetylation/methylation are likely to increase, adding lysines in eEF1A1, KH domain-containing, RNA-binding, signal transduction-associated protein 1 (KHSTAP)1 (also known as Src-associated in mitosis 68 kDa protein Sam68)) and heterogeneous nuclear ribonucleoprotein (hnRNP) D-like to residues in PABP1 [62], whose differential modification were previously postulated to act as ‘molecular switches’.

However, discovery of meK residues lags behind, impeded by its poor digestion with trypsin, its loss during the reversed-phase LC prior to MS (due to the intrinsically basic/hydrophilic nature of meK-containing peptides) and its tendency not to fragment well under collision-induced fragmentation (CID) MS methodologies which necessitates electron-transfer dissociation (ETD)-mediated fragmentation. However significant steps towards the enrichment of

meK-containing proteins/peptides have been made, improving signal-noise ratios (where ‘noise’ is equivalent non-methylated peptides) and enabling robust MS [70]: Firstly, a recombinant affinity agent which detects and enriches mono- and dimeK-containing proteins independently of the surrounding amino acid sequence context [70–72]; consisting of three Lethal(3)malignant brain tumor-like protein 1 (L3MBTL1)-derived malignant brain tumour (MBT) domain repeats]; and secondly, the development of modified *S*-adenosylmethionine (SAM) derivatives, such as propargylic Se-adenosyl-L-selenomethionine (ProSeAM), which allow click chemistry to be deployed to biotinylate mono-, di- or trimethylated lysines, greatly facilitating their enrichment and purification [70,73].

However, it is important to bear in mind that functional studies showing that methylation/acetylation of specific residues are directly regulatory are not straightforward. For instance, PTM cross-talk can result in off-target gain- or loss-of-function, necessitating knowledge of the complete PTM status of wild-type and mutant proteins. Moreover, mutating a specific lysine may inhibit protein function but does not distinguish whether the lysine *per se* or its modification is required for the function under study. Where mutagenesis is used to insert an acK ‘mimic’ (e.g. glutamine) it may indeed mimic some features of acK (e.g. approximate charge) but cannot fully recapitulate the size and charge distribution of the residue, meaning that caution should be exercised in the interpretation of ‘mimic’ effects.

In this regard, the development of techniques that enable methylated or acetylated lysines to be incorporated site-specifically into recombinant proteins was a huge advance for studying PTM function *in vitro*. However, to date, these technologies are not applicable to mammalian cell studies, cannot be used to generate all states of lysine methylation and, in the case of site-specific lysine dimethylation, require harsh denaturation and refolding steps that may be unsuitable for all but small, easily folded proteins/domains [74–76]. Ideally, orthologous systems will be developed to allow the expression of exogenous proteins with defined, site-specific PTMs in mammalian cells but this would appear highly unrealistic, since any exogenous protein would need to be synthesized using the endogenous translational machinery. However, combinations of (1) the directed evolution of aminoacyl tRNA synthetases to utilize unnatural amino acids and/or unnatural tRNAs [74–77], (2) the further development and directed evolution of the orthologous tethered ribosome (Ribo-T) [78] such that it can read quadruplet (or greater) codons [79] and (3) development of an orthologous system for site-specific (quadruplet or greater) codon recoding [similar to the selenocysteine insertion sequence (SECIS) but for recoding only by orthologous ribosomes; [74] and (4) the use of CRISPR-type genome editing to either render endogenous genes amenable to the orthologous apparatus or to insert orthologous decoding-ready exogenous genes; may go some way to enabling the systematic study of the regulation conferred by the vast quantities of novel PTMs currently being identified.

Acknowledgements

We would like to apologize to those whose work we have not had space to cite.

Funding

This work was supported by the Medical Research Council (MRC) grant number [grant number MR/J003069/1 (to N.K.G.)]; and the University of Edinburgh [grant number K606FUN (to T.K.P.B.)].

References

- Lunde, B.M., Moore, C. and Varani, G. (2007) RNA-binding proteins: modular design for efficient function. *Nat. Rev. Mol. Cell Biol.* **8**, 479–490 [CrossRef PubMed](#)
- Dreyfuss, G., Kim, V.N. and Kataoka, N. (2002) Messenger-RNA-binding proteins and the messages they carry. *Nat. Rev. Mol. Cell Biol.* **3**, 195–205 [CrossRef PubMed](#)
- Herschlag, D. (1995) RNA chaperones and the RNA folding problem. *J. Biol. Chem.* **270**, 20871–20874 [CrossRef PubMed](#)
- Semrad, K. (2011) Proteins with RNA chaperone activity: a world of diverse proteins with a common task-impediment of RNA misfolding. *Biochem. Res. Int.* **2011**, 532908 [CrossRef PubMed](#)
- Glisovic, T., Bachorik, J.L., Yong, J. and Dreyfuss, G. (2008) RNA-binding proteins and post-transcriptional gene regulation. *FEBS Lett.* **582**, 1977–1986 [CrossRef PubMed](#)
- Wei, H., Mundade, R., Lange, K.C. and Lu, T. (2014) Protein arginine methylation of non-histone proteins and its role in diseases. *Cell Cycle* **13**, 32–41 [CrossRef PubMed](#)
- Ruben Esse PLIRIT de AHJB and R.C. (2012) Methylation - from DNA, RNA and Histones to Diseases and Treatment. InTech
- Bedford, M.T. and Clarke, S.G. (2009) Protein arginine methylation in mammals: who, what, and why. *Mol. Cell.* **33**, 1–13 [CrossRef PubMed](#)
- Pahlch, S., Zakaryan, R.P. and Gehring, H. (2006) Protein arginine methylation: cellular functions and methods of analysis. *Biochim. Biophys. Acta* **1764**, 1890–1903 [CrossRef PubMed](#)
- Bedford, M.T. and Richard, S. (2005) Arginine methylation: an emerging regulator of protein function. *Mol. Cell* **18**, 263–272 [CrossRef PubMed](#)
- Bardoni, B., Davidovic, L., Bensaid, M. and Khandjian, E.W. (2006) The fragile X syndrome: exploring its molecular basis and seeking a treatment. *Expert Rev. Mol. Med.* **8**, 1–16 [CrossRef PubMed](#)
- Blackwell, E., Zhang, X. and Ceman, S. (2010) Arginines of the RGG box regulate FMRP association with polyribosomes and mRNA. *Hum. Mol. Genet.* **19**, 1314–23 [CrossRef PubMed](#)
- Bechara, E.G., Didiot, M.C., Melko, M., Davidovic, L., Bensaid, M., Martin, P., Castets, M., Pogonec, P., Khandjian, E.W., Moine, H. et al. (2009) A novel function for fragile X mental retardation protein in translational activation. *PLoS Biol.* **7**, e16 [CrossRef PubMed](#)
- Stetler, A., Winograd, C., Sayegh, J., Cheever, A., Patton, E., Zhang, X., Clarke, S. and Ceman, S. (2006) Identification and characterization of the methyl arginines in the fragile X mental retardation protein Fmrp. *Hum. Mol. Genet.* **15**, 87–96 [CrossRef PubMed](#)
- Dolzanskaya, N., Merz, G., Aletta, J.M. and Denman, R.B. (2006) Methylation regulates the intracellular protein-protein and protein-RNA interactions of FMRP. *J. Cell Sci.* **119**, 1933–1946 [CrossRef PubMed](#)
- Denman, R.B. (2002) Methylation of the arginine-glycine-rich region in the fragile X mental retardation protein FMRP differentially affects RNA binding. *Cell. Mol. Biol. Lett.* **7**, 877–883 [PubMed](#)
- Mazroui, R. (2002) Trapping of messenger RNA by fragile X mental retardation protein into cytoplasmic granules induces translation repression. *Hum. Mol. Genet.* **11**, 3007–3017 [CrossRef PubMed](#)
- Kedersha, N. and Anderson, P. (2007) Mammalian stress granules and processing bodies. *Methods Enzymol.* **431**, 61–81 [CrossRef PubMed](#)
- Wang, J., Guo, Y., Chu, H., Guan, Y., Bi, J. and Wang, B. (2013) Multiple Functions of the RNA-binding protein HuR in cancer progression, treatment responses and prognosis. *Int. J. Mol. Sci.* **14**, 10015–10041 [CrossRef PubMed](#)
- Peng, S.S., Chen, C.Y., Xu, N. and Shyu, A.B. (1998) RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J.* **17**, 3461–3470 [CrossRef PubMed](#)
- Vázquez-Chantada, M., Fernández-Ramos, D., Embade, N., Martínez-Lopez, N., Varela-Rey, M., Woodhoo, A., Luka, Z., Wagner, C., Anglim, P.P., Finnell, R.H. et al. (2010) HuR/methyl-HuR and AUF1 regulate the MAT expressed during liver proliferation, differentiation, and carcinogenesis. *Gastroenterology* **138**, 1943–1953 [CrossRef PubMed](#)
- Gubin, M.M., Calaluze, R., Davis, J.W., Magee, J.D., Strouse, C.S., Shaw, D.P., Ma, L., Brown, A., Hoffman, T., Rold, T.L. et al. (2010) Overexpression of the RNA binding protein HuR impairs tumor growth in triple negative breast cancer associated with deficient angiogenesis. *Cell Cycle* **9**, 3337–3346 [CrossRef PubMed](#)
- Suswam, E.A., Nabors, L.B., Huang, Y., Yang, X. and King, P.H. (2005) IL-1beta induces stabilization of IL-8 mRNA in malignant breast cancer cells via the 3' untranslated region: Involvement of divergent RNA-binding factors HuR, KSRP and TIAR. *Int. J. Cancer* **113**, 911–919 [CrossRef PubMed](#)
- Kullmann, M., Göpfert, U., Siewe, B. and Hengst, L. (2002) ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5'UTR. *Genes Dev.* **16**, 3087–3099 [CrossRef PubMed](#)
- Galbán, S., Kuwano, Y., Pullmann, Jr, R., Martindale, J.L., Kim, H.H., Lal, A., Abdelmohsen, K., Yang, X., Dang, Y., Liu, J.O. et al. (2008) RNA-binding proteins HuR and PTB promote the translation of hypoxia-inducible factor 1alpha. *Mol. Cell. Biol.* **28**, 93–107 [CrossRef PubMed](#)
- Srikantan, S., Tominaga, K. and Gorospe, M. (2012) Functional interplay between RNA-binding protein HuR and microRNAs. *Curr. Protein Pept. Sci.* **13**, 372–379 [CrossRef PubMed](#)
- Li, H., Park, S., Kilburn, B., Jelinek, M.A., Henschen-Edman, A., Aswad, D.W., Stallcup, M.R. and Laird-Offringa, I.A. (2002) Lipopolysaccharide-induced methylation of HuR, an mRNA-stabilizing protein, by CARM1. *J. Biol. Chem.* **277**, 44623–44630 [CrossRef PubMed](#)
- Fan, X.C. and Steitz, J.A. (1998) HNS, a nuclear-cytoplasmic shuttling sequence in HuR. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15293–15298 [CrossRef PubMed](#)
- Pang, L., Tian, H., Chang, N., Yi, J., Xue, L., Jiang, B., Gorospe, M., Zhang, X. and Wang, W. (2013) Loss of CARM1 is linked to reduced HuR function in replicative senescence. *BMC Mol. Biol.* **14**, 15 [CrossRef PubMed](#)
- Yu, M.C. (2011) The role of protein arginine methylation in mRNP dynamics. *Mol. Biol. Int.* **2011**, 163827 [CrossRef PubMed](#)
- Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V. and Mann, M. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325**, 834–840 [CrossRef PubMed](#)
- Choudhary, C., Weinert, B.T., Nishida, Y., Verdin, E. and Mann, M. (2014) The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat. Rev. Mol. Cell Biol.* **15**, 536–550 [CrossRef PubMed](#)
- Yang, X.-J. (2004) The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res.* **32**, 959–976 [CrossRef PubMed](#)
- Sadoul, K., Wang, J., Diagouraga, B. and Khochbin, S. (2011) The tale of protein lysine acetylation in the cytoplasm. *J. Biomed. Biotechnol.* **2011**, 970382 [CrossRef PubMed](#)
- Yang, X.-J. and Seto, E. (2008) Lysine acetylation: crosstalk with other posttranslational modifications. *Mol. Cell.* **31**, 449–461 [CrossRef PubMed](#)
- Kaehlcke, K., Dorr, A., Hetzer-Egger, C., Kiermer, V., Henklein, P., Schnoelzer, M., Lorent, E., Cole, P.A., Verdin, E. and Ott, M. (2003) Acetylation of Tat defines a CyclinT1-independent step in HIV transactivation. *Mol. Cell.* **12**, 167–176 [CrossRef PubMed](#)
- Sol, E.M., Wagner, S.A., Weinert, B.T., Kumar, A., Kim, H.S., Deng, C.X. and Choudhary, C. (2012) Proteomic investigations of lysine acetylation identify diverse substrates of mitochondrial deacetylase sirt3. *PLoS One* **7**, e50545 [CrossRef PubMed](#)
- Schölz, C., Weinert, B.T., Wagner, S.A., Beli, P., Miyake, Y., Qi, J., Jensen, L.J., Streicher, W., McCarthy, A.R. and Westwood, N.J. (2015) Acetylation site specificities of lysine deacetylase inhibitors in human cells. *Nat. Biotechnol.* **33**, 415–423 [CrossRef PubMed](#)
- Yajima, M. and Wessel, G.M. The multiple hats of Vasa: its functions in the germline and in cell cycle progression. *Mol. Reprod. Dev.* **78**, 861–867 [CrossRef](#)
- Nagamori, I., Cruickshank, V.A. and Sassone-Corsi, P. (2011) Regulation of an RNA granule during spermatogenesis: acetylation of MVH in the chromatoid body of germ cells. *J. Cell Sci.* **124**, 4346–4355 [CrossRef PubMed](#)
- Kotaja, N. and Sassone-Corsi, P. (2007) The chromatoid body: a germ-cell-specific RNA-processing centre. *Nat. Rev. Mol. Cell Biol.* **8**, 85–90 [CrossRef PubMed](#)

- 42 Kotaja, N., Bhattacharyya, S.N., Jaskiewicz, L., Kimmins, S., Parvinen, M., Filipowicz, W. and Sassone-Corsi, P. (2006) The chromatoid body of male germ cells: similarity with processing bodies and presence of Dicer and microRNA pathway components. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 2647–2652 [CrossRef PubMed](#)
- 43 Kuramochi-Miyagawa, S., Watanabe, T., Gotoh, K., Takamatsu, K., Chuma, S., Kojima-Kita, K., Shiromoto, Y., Asada, N., Toyoda, A. and Fujiyama, A. (2010) MVH in piRNA processing and gene silencing of retrotransposons. *Genes Dev.* **24**, 887–892 [CrossRef PubMed](#)
- 44 Tanaka, S.S., Toyooka, Y., Akasu, R., Katoh-Fukui, Y., Nakahara, Y., Suzuki, R., Yokoyama, M. and Noce, T. (2000) The mouse homolog of *Drosophila* Vasa is required for the development of male germ cells. *Genes Dev.* **14**, 841–853 [PubMed](#)
- 45 Li, Y. and Guan, X.-Y. (2014) EIF5A2 promotes tumor metastasis and angiogenesis. *Sci. Proc.* **1**, [CrossRef](#)
- 46 Mathews, M.B. and Hershey, J.W.B. (2015) The translation factor eIF5A and human cancer. *Biochim. Biophys. Acta* **1849**, 836–844 [CrossRef PubMed](#)
- 47 Barjaktarovic, Z., Kempf, S.J., Sriharshan, A., Merl-Pham, J., Atkinson, M.J. and Tapio, S. (2015) Ionizing radiation induces immediate protein acetylation changes in human cardiac microvascular endothelial cells. *J. Radiat. Res.* **56**, 623–632 [CrossRef PubMed](#)
- 48 Ishfaq, M., Maeta, K., Maeda, S., Natsume, T., Ito, A. and Yoshida, M. (2012) The role of acetylation in the subcellular localization of an oncogenic isoform of translation factor eIF5A. *Biosci. Biotechnol. Biochem.* **76**, 2165–2167 [CrossRef PubMed](#)
- 49 Ishfaq, M., Maeta, K., Maeda, S., Natsume, T., Ito, A. and Yoshida, M. (2012) Acetylation regulates subcellular localization of eukaryotic translation initiation factor 5A (eIF5A). *FEBS Lett.* **586**, 3236–3241 [CrossRef PubMed](#)
- 50 Kwong, L.K., Uryu, K., Trojanowski, J.Q. and Lee, V.M.-Y. (2008) TDP-43 proteinopathies: neurodegenerative protein misfolding diseases without amyloidosis. *Neurosignals* **16**, 41–51 [CrossRef PubMed](#)
- 51 Hasegawa, M., Arai, T., Nonaka, T., Kametani, F., Yoshida, M., Hashizume, Y., Beach, T.G., Buratti, E., Baralle, F., Morita, M. et al. (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann. Neurol.* **64**, 60–70 [CrossRef PubMed](#)
- 52 Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M. et al. (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130–133 [CrossRef PubMed](#)
- 53 Lee, E.B., Lee, V.M.-Y. and Trojanowski, J.Q. (2012) Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. *Nat. Rev. Neurosci.* **13**, 38–50
- 54 Cohen, T.J., Hwang, A.W., Restrepo, C.R., Yuan, C.-X., Trojanowski, J.Q. and Lee, V.M.Y. (2015) An acetylation switch controls TDP-43 function and aggregation propensity. *Nat. Commun.* **6**, 5845 [CrossRef PubMed](#)
- 55 Igaz, L.M., Kwong, L.K., Chen-Plotkin, A., Winton, M.J., Unger, T.L., Xu, Y., Neumann, M., Trojanowski, J.Q. and Lee, V.M. (2009) Expression of TDP-43 C-terminal fragments *in vitro* recapitulates pathological features of TDP-43 proteinopathies. *J. Biol. Chem.* **284**, 8516–8524 [CrossRef PubMed](#)
- 56 Hamamoto, R., Saloura, V. and Nakamura, Y. (2015) Critical roles of non-histone protein lysine methylation in human tumorigenesis. *Nat. Rev. Cancer* **15**, 110–124 [CrossRef PubMed](#)
- 57 Lanouette, S., Mongeon, V., Figeys, D. and Couture, J.-F. (2014) The functional diversity of protein lysine methylation. *Mol. Syst. Biol.* **10**, 724 [CrossRef PubMed](#)
- 58 Huang, J. and Berger, S.L. (2008) The emerging field of dynamic lysine methylation of non-histone proteins. *Curr. Opin. Genet. Dev.* **18**, 152–158 [CrossRef PubMed](#)
- 59 Zhang, X., Wen, H. and Shi, X. (2012) Lysine methylation: beyond histones. *Acta Biochim. Biophys. Sin.* **44**, 14–27 [CrossRef](#)
- 60 Clarke, S.G. (2013) Protein methylation at the surface and buried deep: thinking outside the histone box. *Trends Biochem. Sci.* **38**, 243–252 [CrossRef PubMed](#)
- 61 Zhang, X., Huang, Y. and Shi, X. (2015) Emerging roles of lysine methylation on non-histone proteins. *Cell. Mol. Life Sci.*, in the press
- 62 Brook, M., McCracken, L., Reddington, J.P., Lu, Z.-L., Morrice, N.A. and Gray, N.K. (2012) The multifunctional poly(A)-binding protein (PABP) 1 is subject to extensive dynamic post-translational modification, which molecular modelling suggests plays an important role in co-ordinating its activities. *Biochem. J.* **441**, 803–812 [CrossRef PubMed](#)
- 63 Polevoda, B. and Sherman, F. (2007) Methylation of proteins involved in translation. *Mol. Microbiol.* **65**, 590–606 [CrossRef PubMed](#)
- 64 Mateyak, M.K. and Kinzy, T.G. (2010) eEF1A: thinking outside the ribosome. *J. Biol. Chem.* **285**, 21209–21213 [CrossRef PubMed](#)
- 65 Vermillion, K.L., Lidberg, K.A. and Gammill, L.S. (2014) Cytoplasmic protein methylation is essential for neural crest migration. *J. Cell Biol.* **204**, 95–109 [CrossRef PubMed](#)
- 66 Cao, X.-J., Arnaudo, A.M. and Garcia, B.A. (2013) Large-scale global identification of protein lysine methylation *in vivo*. *Epigenetics* **8**, 477–485 [CrossRef PubMed](#)
- 67 Bremang, M., Cuomo, A., Agresta, A.M., Stugiewicz, M., Spadotto, V. and Bonaldi, T. (2013) Mass spectrometry-based identification and characterisation of lysine and arginine methylation in the human proteome. *Mol. Biosyst.* **9**, 2231–2247 [CrossRef PubMed](#)
- 68 Wu, Z., Cheng, Z., Sun, M., Wan, X., Liu, P., He, T., Tan, M. and Zhao, Y. (2015) A chemical proteomics approach for global analysis of lysine monomethylome profiling. *Mol. Cell. Proteomics* **14**, 329–339 [CrossRef PubMed](#)
- 69 Sylvestersen, K.B., Horn, H., Jungmichel, S., Jensen, L.J. and Nielsen, M.L. (2014) Proteomic analysis of arginine methylation sites in human cells reveals dynamic regulation during transcriptional arrest. *Mol. Cell. Proteomics* **13**, 2072–2088 [CrossRef PubMed](#)
- 70 Carlson, S.M. and Gozani, O. (2014) Emerging technologies to map the protein methylome. *J. Mol. Biol.* **426**, 3350–3362 [CrossRef PubMed](#)
- 71 Moore, K.E. and Gozani, O. (2014) An unexpected journey: lysine methylation across the proteome. *Biochim. Biophys. Acta* **1839**, 1395–1403 [CrossRef PubMed](#)
- 72 Moore, K.E., Carlson, S.M., Camp, N.D., Cheung, P., James, R.G., Chua, K.F., Wolf-Yadlin, A. and Gozani, O. (2013) A general molecular affinity strategy for global detection and proteomic analysis of lysine methylation. *Mol. Cell.* **50**, 444–456 [CrossRef PubMed](#)
- 73 Shimazu, T., Barjau, J., Sohtome, Y., Sodeoka, M. and Shinkai, Y. (2014) Selenium-based S-adenosylmethionine analog reveals the mammalian seven-beta-strand methyltransferase METTL10 to be an EF1A1 lysine methyltransferase. *PLoS One* **9**, e105394 [CrossRef PubMed](#)
- 74 Neumann, H., Peak-Chew, S.Y. and Chin, J.W. (2008) Genetically encoding N(epsilon)-acetyllysine in recombinant proteins. *Nat. Chem. Biol.* **4**, 232–234 [CrossRef PubMed](#)
- 75 Nguyen, D.P., Garcia Alai, M.M., Virdee, S. and Chin, J.W. (2010) Genetically directing ϵ -N, N-dimethyl-L-lysine in recombinant histones. *Chem. Biol.* **17**, 1072–1076 [CrossRef PubMed](#)
- 76 Neumann, H., Hancock, S.M., Buning, R., Routh, A., Chapman, L., Somers, J., Owen-Hughes, T., van Noort, J., Rhodes, D. and Chin, J.W. (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. *Mol. Cell.* **36**, 153–163 [CrossRef PubMed](#)
- 77 Nguyen, D.P., Garcia Alai, M.M., Kapadnis, P.B., Neumann, H. and Chin, J.W. (2009) Genetically encoding N(epsilon)-methyl-L-lysine in recombinant histones. *J. Am. Chem. Soc.* **131**, 14194–14195 [CrossRef PubMed](#)
- 78 Orelle, C., Carlson, E.D., Szal, T., Florin, T., Jewett, M.C. and Mankin, A.S. (2015) Protein synthesis by ribosomes with tethered subunits. *Nature* **524**, 119–124 [CrossRef PubMed](#)
- 79 Wang, K., Schmied, W.H. and Chin, J.W. (2012) Reprogramming the genetic code: from triplet to quadruplet codes. *Angew. Chem. Int. Ed. Engl.* **51**, 2288–2297 [CrossRef PubMed](#)
- 80 Mertins, P., Qiao, J.W., Patel, J., Udeshi, N.D., Clauser, K.R., Mani, D.R., Burgess, M.W., Gillette, M.A., Jaffe, J.D. and Carr, S.A. (2013) Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat. Methods* **10**, 634–637 [CrossRef PubMed](#)
- 81 Rardin, M.J., Newman, J.C., Held, J.M., Cusack, M.P., Sorensen, D.J., Li, B., Schilling, B., Mooney, S.D., Kahn, C.R., Verdin, E. et al. (2013) Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 6601–6606 [CrossRef PubMed](#)
- 82 Svinikina, T., Gu, H., Silva, J.C., Mertins, P., Qiao, J., Fereshetian, S., Jaffe, J.D., Kuhn, E., Udeshi, N.D. and Carr, S.A. (2015) Deep, quantitative coverage of the lysine acetylome using novel anti-acetyl-lysine antibodies and an optimized proteomic workflow. *Mol. Cell. Proteomics* **14**, 2429–2440 [CrossRef PubMed](#)
- 83 Chen, Y., Zhao, W., Yang, J.S., Cheng, Z., Luo, H., Lu, Z., Tan, M., Gu, W. and Zhao, Y. (2012) Quantitative acetylome analysis reveals the roles of SIRT1 in regulating diverse substrates and cellular pathways. *Mol. Cell. Proteomics* **11**, 1048–1062 [CrossRef PubMed](#)
- 84 Lundby, A., Lage, K., Weinert, B.T., Bekker-Jensen, D.B., Secher, A., Skovgaard, T., Kelstrup, C.D., Dmytriiev, A., Choudhary, C., Lundby, C. et al. (2012) Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. *Cell Rep.* **2**, 419–431 [CrossRef PubMed](#)

- 85 Weinert, B.T., Schölz, C., Wagner, S.A., Iesmantavicius, V., Su, D., Daniel, J.A. and Choudhary, C. (2013) Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. *Cell Rep.* **4**, 842–851 [CrossRef PubMed](#)
- 86 Wu, C.H., Weng, M.H., Chang, H.C., Li, J.H. and Cheng, R.P. (2014) Effect of each guanidinium group on the RNA recognition and cellular uptake of Tat-derived peptides. *Bioorg. Med. Chem.* **22**, 3016–3020 [CrossRef PubMed](#)
- 87 Shaw, P.G., Chaerkady, R., Zhang, Z., Davidson, N.E. and Pandey, A. (2011) Monoclonal antibody cocktail as an enrichment tool for acetylome analysis. *Anal. Chem.* **83**, 3623–3626 [CrossRef PubMed](#)
- 88 Beli, P., Lukashchuk, N., Wagner, S.A., Weinert, B.T., Olsen, J.V., Baskcomb, L., Mann, M., Jackson, S.P. and Choudhary, C. (2012) Proteomic investigations reveal a role for RNA processing factor THRAP3 in the DNA damage response. *Mol. Cell.* **46**, 212–225 [CrossRef PubMed](#)
- 89 Simon, G.M., Cheng, J. and Gordon, J.I. (2012) Quantitative assessment of the impact of the gut microbiota on lysine epsilon-acetylation of host proteins using gnotobiotic mice. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11133–11138 [CrossRef PubMed](#)
- 90 Sasaki, K., Suzuki, A., Kagatsume, S., Ono, M., Matsuzawa, K., Taguchi, Y. and Kurihara, Y. (2012) Acetylation of Prp K150 regulates the subcellular localization. *Gene* **491**, 13–19 [CrossRef PubMed](#)
- 91 Hebert, A.S., Dittenhafer-Reed, K.E., Yu, W., Bailey, D.J., Selen, E.S., Boersma, M.D., Carson, J.J., Tonelli, M., Balloon, A.J., Higbee, A.J. et al. (2013) Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Mol. Cell* **49**, 186–199 [CrossRef PubMed](#)
- 92 Guo, A., Gu, H., Zhou, J., Mulhern, D., Wang, Y., Lee, K.A., Yang, V., Aguiar, M., Kornhauser, J., Jia, X. et al. (2014) Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. *Mol. Cell. Proteomics* **13**, 372–387 [CrossRef PubMed](#)
- 93 Bryson, B.D. and White, F.M. (2015) Quantitative profiling of lysine acetylation reveals dynamic crosstalk between receptor tyrosine kinases and lysine acetylation. *PLoS One* **10**, e0126242 [CrossRef PubMed](#)
- 94 Masri, S., Patel, V.R., Eckel-Mahan, K.L., Peleg, S., Forne, I., Ladurner, A.G., Baldi, P., Imhof, A. and Sassone-Corsi, P. (2013) Circadian acetylome reveals regulation of mitochondrial metabolic pathways. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 3339–3344 [CrossRef PubMed](#)
- 95 Witalison, E.E., Thompson, P.R. and Hofseth, L.J. (2015) Protein Arginine deiminases and associated citrullination: physiological functions and diseases associated with dysregulation. *Curr. Drug Targets* **16**, 700–710 [CrossRef PubMed](#)
- 96 Wang, S. and Wang, Y. (2013) Peptidylarginine deiminases in citrullination, gene regulation, health and pathogenesis. *Biochim. Biophys. Acta* **1829**, 1126–1135 [CrossRef PubMed](#)
- 97 Chang, B., Chen, Y., Zhao, Y. and Bruick, R.K. (2007) JMJD6 is a histone arginine demethylase. *Science* **318**, 444–447 [CrossRef PubMed](#)
- 98 Del Rizzo, P.A. and Trievel, R.C. (2014) Molecular basis for substrate recognition by lysine methyltransferases and demethylases. *Biochim. Biophys. Acta* **1839**, 1404–1415 [CrossRef PubMed](#)

Received 11 August 2015
doi:10.1042/BST20150172