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

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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 coordinating 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 of gene products. Recent findings have emphasised 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 (summarised in [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, localisation, translation and decay (reviewed in [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 post-translational modification (PTM), of which phosphorylation is the best-characterised example. More recently 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 localisation, RNA-binding activity, protein-protein interactions or combinations thereof[5]. Using selected examples of mammalian cytoplasmic PTRPs we summarise the functional roles of arginine methylation, the most studied non-phosphorylation PTM in this regard. Emerging roles of non-histone lysine acetylation and methylation and the putative utilisation 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) proteomics (Table 1) that point to the potential untapped regulatory consequences of such PTMs in the cytoplasmic coordination of mRNA utilisation.

Arginine Methylation

Arginine methylation of non-histone proteins is an established area which has been reviewed extensively [6,7], albeit not with respect to regulation of mRNA fate. Briefly, arginine methylation occurs predominantly in arginine-glycine (RG)-rich regions, and is catalysed by protein arginine methyltransferases (PRMTs). Addition of each methyl group [See 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 subcellular localisation (reviewed in[6,9]) which has implications for many cellular processes, and can result in pathogenesis [6,8,10] (see Figure 2). Here, we give two examples of the varied effects of arginine methylation on cytoplasmic PTRP activities.

Fragile X mental retardation protein (FMRP). FMRP is a nucleocytoplasmic shuttling protein whose absence is associated with Fragile X syndrome (reviewed in[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 multifunctionality 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 FRMP heterodimerisation with its paralogs FXR1P and FXR2P, but not FMRP homodimerisation[12,15,16], although the functional significance of this remains unclear. Furthermore, whilst AdOx-treatment did not alter FMRP recruitment to arsenite-induced stress granules (SGs)[17], which are 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 whilst 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 (HuR). ELAVL1 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' untranslated regions (3'UTR) (reviewed in[19]) to stabilise [20], destabilise [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 (reviewed in[26]) with these functions not being mutually exclusive and potentially operating in concert.

In cells ELAVL1 can be methylated at Arg^{217} [27], which lies within the hinge region of its nucleocytoplasmic shuttling sequence that binds nuclear export cofactors[28]. Arg^{217} dimethylation is enhanced during liposaccharide-mediated stimulation of macrophage and leukaemia cell lines concomitant to its relocalisation to the cytoplasm. However, whilst PRMT4-dependent Arg^{217} methylation is proposed to regulate both export and nuclear reimport of cytoplasmic ELAVL1, no direct role in this process is yet demonstrated. Arg^{217} methylation also modulates mRNA stability with unmethylated ELAVL1 stabilising and Arg^{217} methylated ELAVL1 destabilising MET2A 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 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 TNFα) 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 lysine acetylation (acK) and the effector enzymes that add/remove this PTM (Figure 1) is derived from studies of histone function[31,32]. Acetylation neutralises 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) (reviewed in[33]) but, to date, very few *bona fide* cytoplasmic substrates are identified. Despite this there has been a recent explosion in the numbers of putatively identified lysine acetylations 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 lysine acetylation is now considered as pervasive as protein phosphorylation. Three examples of PTRPs regulated by lysine acetylation are described below.

Dead-box polypeptide 4 (DDX4) (also called VASA (human) and Mouse VASA Homolog (MVH)). In mammals, DDX4 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 localisation, DDX4 interacts with several RNA-induced silencing complex (RISC) components [40–42] and is implicated translational repression mediated by small RNAs (i.e. miRNAs and piRNAs) [42–44]. Lys^{405} acetylation of DDX4 at stages IV to 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 characterised testis- and brain-restricted eIF5A2 (reviewed in [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 Lys^{50} (hypK^{50}) leads to its nuclear export and cytoplasmic retention [47–49]. Whilst hypusination is irreversible, eIF5A1 can also be acetylated at Lys^{47}, which antagonises Lys^{50} hypusination and prevents its nuclear export, thereby providing a mechanism to regulate eIF5A1 subcellular localisation. Furthermore, both Lys^{50} hypusination and retention of the positive charge state of Lys^{47} are essential for its translational functions [48,49] and Lys47 acetylation therefore provides a fail-safe mechanism to abrogate eIF5A-dependent translation should any escape into the cytoplasm.

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

Transactive response (TAR) DNA-binding protein (TDP)-43. TDP-43 is a nucleocytoplasmic shuttling protein which is predominantly nuclear localised 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 (reviewed in [53]). Lys$^{145/192}$ of the RNA-recognition motif (RRM) of TDP-43 can be acetylated and TDP-43 acetylation mimic mutants (e.g. Lys-Gln) 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 subcellular localisation, although it is unclear whether these two processes are distinct or linked, and TDP-43 lysine acetylation may be dysregulated by environmental stress. Lys^{145} acetylation is detected in ALS patient brain/spinal cord inclusions but not those from FTLD-TDP, in whom TDP-43 is C-terminally truncated and lacks Lys145 [55], therefore it is unclear whether TDP-43 lysine acetylation is directly involved in the pathogenesis of ALS proteinopathy.

Lysine methylation

Like acetylation, lysine mono-, di- and tri-methylation (Figure 1) are a well-established PTMs with profound regulatory functions in chromatin remodelling and transcription (reviewed in [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-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 (reviewed elsewhere [57,59]).

Whereas lysine acetylation alters net charge, methylation does not and in fact, trimethylation actually stabilises the positive charge of this sidechain (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 likely 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-alpha 1 (eEF1A1)* which is one of the most repeatedly detected acetylated/methylated proteins in proteomics studies (see Table 1), most likely due to its very high cellular concentration, with 21 high-probability acK residues and 8 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 (reviewed in [63]). eEF1A1 also has non-canonical functions in cytoskeletal organisation (reviewed in [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. Whilst, the functions of eEF1A1 PTMs are undetermined, trimethylation of eEF1A1 Lys^{318} was recently documented and is one of several meK residues implicated in chick neural crest migration[65]. Given the conservation of this PTM from yeast (Lys^{316}), it is tempting to speculate that eEF1A1 lysine methylation may have similar relevance in mammals.

Whilst 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 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 to 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).

Realising 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 (see Supplemental Table 1 for a more extensive table including PTMs detected in single studies). Whilst, the vast majority of these PTMs are unstudied, collectively this 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, KHSTAP1 (Sam68) and hnRNPD-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 liquid chromatography 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 L3MBTL1-derived malignant brain tumour (MBT) domain repeats (3xMBT)); 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 [73] (reviewed in [70]).

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 crosstalk 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 is 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 synthesised using the endogenous translational machinery. However, combinations of *1)* the directed evolution of aminoacyl tRNA synthetases to utilise 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.

Protein	acK residue	meK residue	Protein	acK residue	meK residue	Protein	acK residue	meK residue	Protein	acK residue	meK residue	Protein	acK residue	meK residue
CNBP	8[38,80,81]		eEF1A1 (cont'd)	395[31,38,80,82,83]		eIF4B (cont'd)	557[83,84]		hnRNP C1/2 (cont'd)	39[31,38,80,82,83]		PABP1	78[18,80,84,85]	
CNBP	103[80,81]			408[38,83]	408[67]		586[31,84]				98[86]		95[62]	
DAZAP1	59[38,83]			439[31,38,80,82,83,85,87–89]			592[37,38]			144 (C1)[80,84]			104[31,37,38,80,82–85]	
DAZAP1	103[31,82]		eIF1b	109[37,83]		eIF4E	119[17,83–85]			203 (C1)[31,38,80]			108[31,38,80,82,84,88]	
DAZAP1	150[90]		eIF2α	141[31,37,38,80,83–85]		eIF4G1	190 [A + 8][§][37,38,80,84,85]			204 (C2)[83,84]			157[82,84]	
DDX1	117[80,91]		eIF2Bα		145[86]		597[83,85]			216[37,38,82]			188[62,84,86]	188[62]
DDX1	239[31,37,38,85]		eIF3A		2 [2][86]		950[37,80,88]			219[80,82]			259[31,37,38,84]	
DDX1	268[31,83]			68[31,37,38,80,82–85]				1026[86]	hnRNPD (Isoforms 1 + 3)	72 [1-4][80]			299[37,38,62,80,85]	299[62]
DDX1	281[31,37,38,80,83,85]			538[37,38]		eIF4H	80[80,82,84]			114[37,38,80,82,83,85]			312[62]	312[62]
DDX1	596[37,38,84,85]				632[66]		123[18,84]			119[83]			361[62,83]	361[62]
eEF1A1		36[69,92]			633[66]			130[86]		129[37,38,80,82]			606[62]	606[62]
	41[38,80,82,83,85,89,91]				634[66]		214[82,84]			153[80]			620[37,38,83]	
	44[38,80,82,83,85,88]		eIF4A1	54[37,82–84]			245[31,37,38,80,82,84]			165[31,38,80,85]		PAIP2	123[38,80]	
		51[92]		68[38,83]		eIF5A-1	27 [1][31,84]			176[82,84]		PARN	220[31,38,85]	
	55[82]	55[67,92]		118[31,37,38,83,85]			47 [1][31,37,83,84,93]			197[37,38,80,83,85]			499[31,38,80]	
		62[92]		146[37,38,80,83,85]			49 [1] or 69 [2][38,80,82,85]			218[38,85]		PTB1	436[80,85]	
	79[87]	79[67,92]		174[31,37,38,82,84,85]			77[2][31,37,38,80,82,85,88]			231[38,83]		KHSTAP1 (Sam68)	96[37,38,84,85]	
	84[31,87,88]	84[67,92]		193[37,38,80,82–85,91]			97[2][38,80,82]			243[37,80,82]			165[37,82]	
	146[38,80,82,83,85,88]			238[38,80,83–85]			151 [2][37,38,80,85]			251[37,38,82,83]			169[31,38,80,88]	
	154[38,67,83]			291[31,37,38,84]		eRF3A	345 [2][38,80]		hnRNPD-like	76[82,86]	76		175[31,37,38,80,81,83–85,88]	
	165[38,82,83,85,89]	165[67,69,92]		309[31,37,38,80,82,84,85]			377 [2][37,38]			137[38,84]			194*[82]	194*[66]
	172[31,38,80,82,83,85,88,89,91]	172[69]		381[37,38,80,85]			391 [2][82,83]			161[80,82]	161[66]			200[66]
	179[80–83,85,88,89,91,94]		eIF4B	177[37,38]			627 [2][37,38]			204[37,38,82]		SERBP1	68[31,37,38,80,84]	
	180[31,82]			194[37,38,84]		EWSR1	438 [3][37,38,80,84,85]			216[37,38,80,84,88]			102[38,84]	
	212[38,85]			223[37,38]			444 [5][37,38,80,82,84,88]		ELAVL1	55[38,81]			122[31,37,38,80,84,85,88]	
	244[38,83]			365[37,38,83,84]			646 [5][82,84]		KSRP		71[86]		140[31,80,82,84,88]	
	255[38,80,82,83,85,88]			395[84]			649 [5][18,82,84]			87[37,83,84]			211[31,37,38,84]	
	273[31,38,80,82,83,85,89]				437[86]	FUS	327 (mouse)[81,84]			122[82,84]			236[37,38,85]	
	318[38,80,82]	318[42,67,92]		447 [2][37,38,85]			332[38,80,82]			169[31,80,84]		TAF15	297[37,88]	
	392[31,38,80,82,83,85,89,91,94]					hnRNPC1/2	8[38,80]			359[83,84]				

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)** Lysine acetyltransferase (KATs) *1)* acetylate the epsilon nitrogen of lysine residues to create acetyllysine [32]. This is reversed *2)* by lysine deacetylases (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 lysine methyltransferases (KMTs). Not all characterised KMTs can catalyse all methylation states. Lysine demethylation *4)* is catalysed by lysine demethylases (KDMTs) [57,59]. The lysine-<u>s</u>pecific <u>d</u>emethylase (LSD) lysine demethylases 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 localisation.* ELAVL1 is a multifunctional 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 modification at one residue can affect another.* eIF5A is hypusinated at Lys^{50} enabling its export to the cytoplasm where it functions in mRNA translation/stability (i-ii). However, Lys^{47} acetylation by PCAF antagonises hypusination retaining eIF5A in the nucleus. Acetylation may be reversed by HDAC6/SIRT2 **C)** *Protein-protein* and **D)** *RNA-protein interactions are PTM sensitive.* **C)** Non-methylated FMRP binds paralogs FXR1P and FXR2P inefficiently. Methylation by PRMT4 enhances FMRP heterodimerisation with FXR1P and FXR2P, but has no apparent effect on FMRP homodimerisation, 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 (ORF2) is unaffected. Red and green arrows, nuclear export and import, respectively; wavy lines mRNA; ORF=open reading frame; Me =methylation; Ac=acetylation; hyp = hypusination; PRTM4= protein arginine methyltransferase 4; miRNA = miRNA-mediated translation repression complex; ARE = AU-rich element. Blue arrows represent interactions, with broader blue arrows representing enhanced interactions and dotted blue lines with a cross representing impeded interactions.

Table 1. Collated high-confidence post-transcriptional effector protein lysine acetylations 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 Lys[194] is not conserved from mouse to human. An expanded version of this data can be viewed in Supplemental Table 1.

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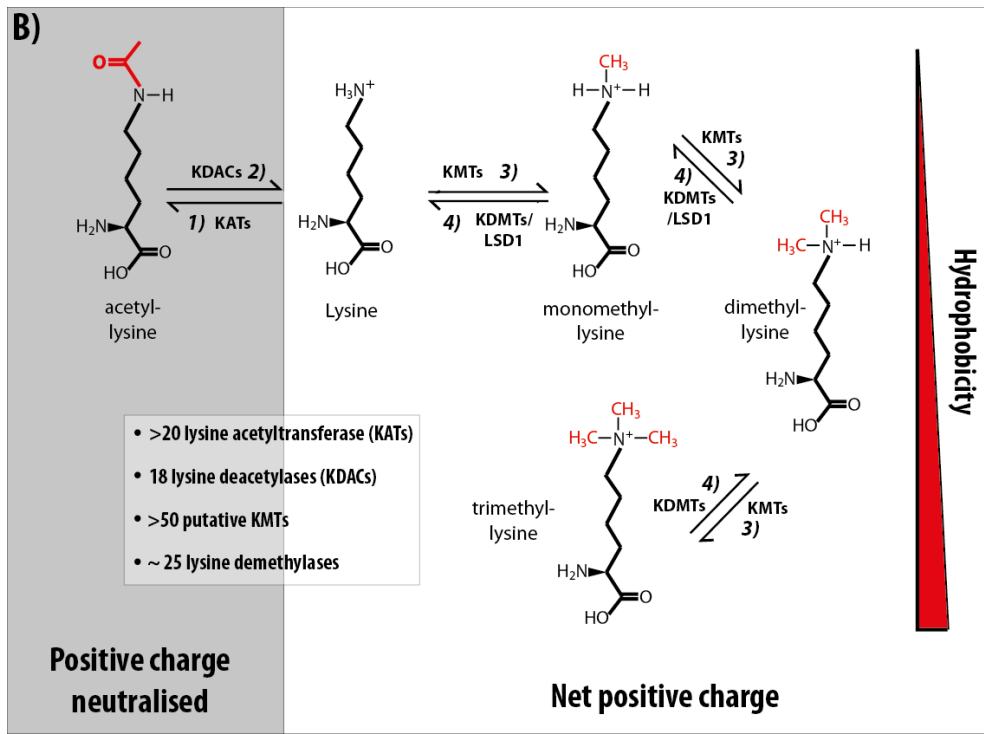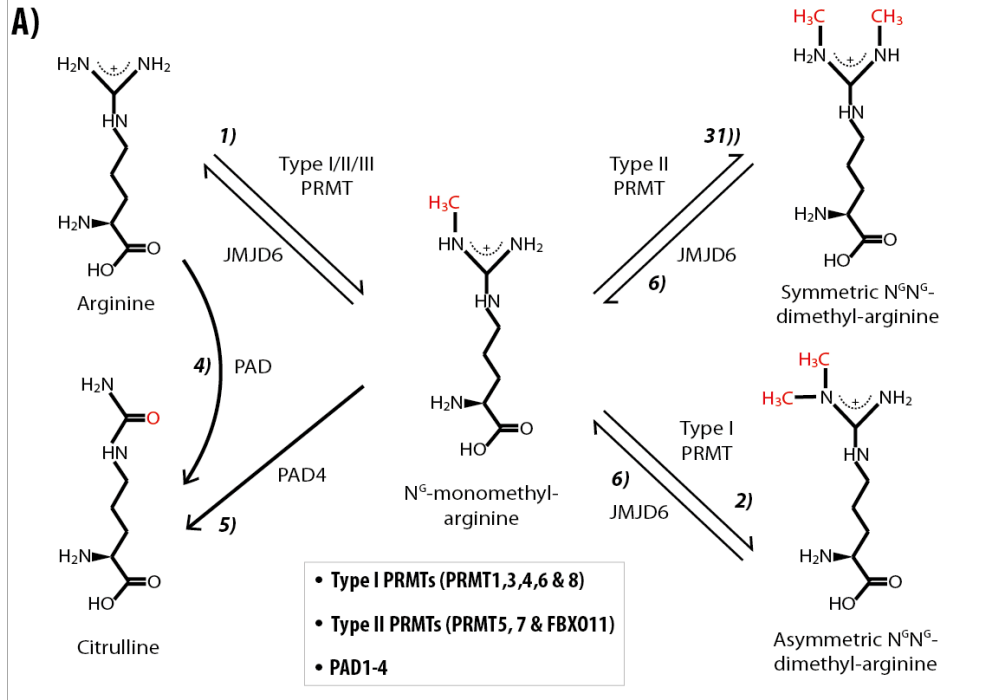
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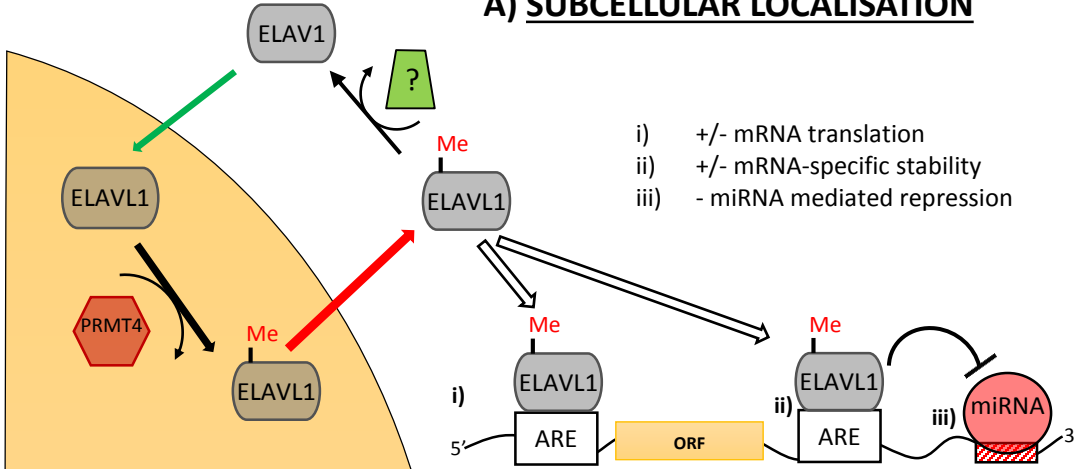
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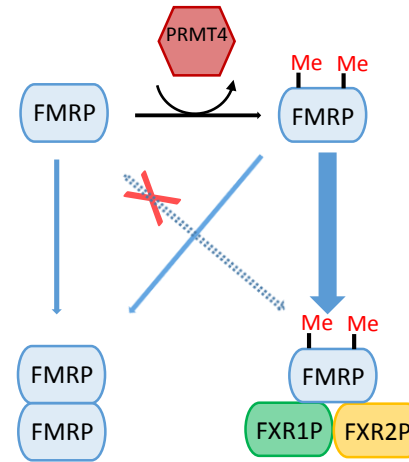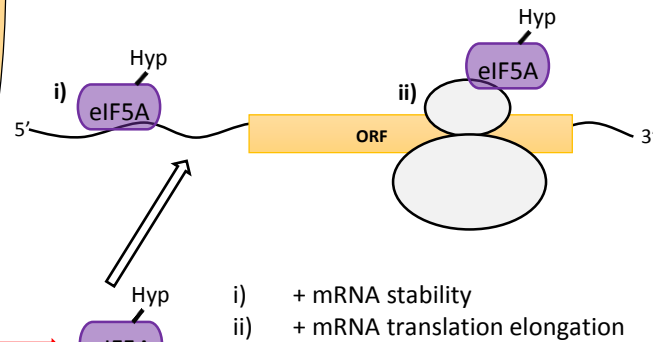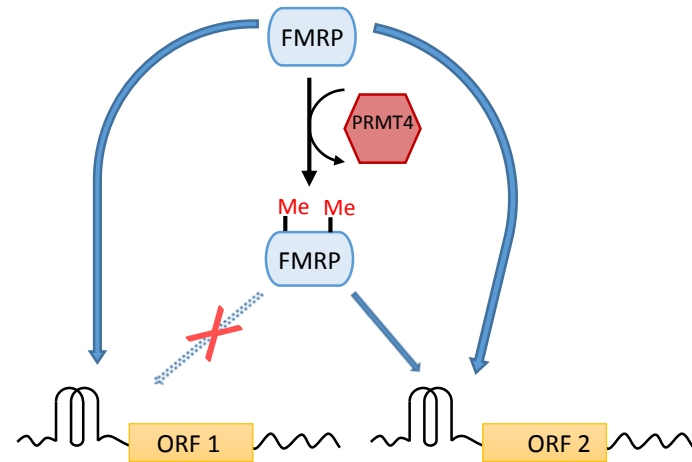
CYTOPLASM

A) SUBCELLULAR LOCALISATION

i)   +/- mRNA translation
ii)   +/- mRNA-specific stability
iii) - miRNA mediated repression

ELAV1

?

Me ELAVL1

Me ELAVL1

PRMT4

Me ELAVL1

i) Me ELAVL1 5' ARE ORF

ii) Me ELAVL1 ARE iii) miRNA 3'

NUCLEUS

Ac eIF5A

PCAF   HDAC6/ SIRT2

eIF5A

Hyp eIF5A

B) FUNCTIONAL CROSSTALK AND HIERARCHY

i) Hyp eIF5A 5' ORF

ii) Hyp eIF5A 3'

i) + mRNA stability
ii) + mRNA translation elongation

C) PROTEIN-PROTEIN INTERACTIONS

PRMT4

FMRP

Me Me FMRP

FMRP   FMRP

Me Me FMRP   FXR1P FXR2P

D) RNA-BINDING ACTIVITY

FMRP

PRMT4

Me Me FMRP

ORF 1   ORF 2