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REVIEW

Cellular consequences of arginine methylation

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Abstract

Arginine methylation is a ubiquitous post-translational modification. Three predominant types of arginine-guanidino methylation occur in Eukarya: mono (Rme1/MMA), symmetric (Rme2s/SDMA), and asymmetric (Rme2a/ADMA). Arginine methylation frequently occurs at sites of protein–protein and protein–nucleic acid interactions, providing specificity for binding partners and stabilization of important biological interactions in diverse cellular processes. Each methylarginine isoform—catalyzed by members of the protein arginine methyltransferase family, Type I (PRMT1-4,6,8) and Type II (PRMT5,9)—has unique downstream consequences. Methylarginines are found in ordered domains, domains of low complexity, and in intrinsically disordered regions of proteins—the latter two of which are intimately connected with biological liquid–liquid phase separation. This review highlights discoveries illuminating how arginine methylation affects genome integrity, gene transcription, mRNA splicing and mRNP biology, protein translation and stability, and phase separation. As more proteins and processes are found to be regulated by arginine methylation, its importance for understanding cellular physiology will continue to grow.

Keywords Protein arginine methyltransferase · Histones · Ribonucleoprotein · Crosstalk · Liquid–liquid phase separation

Introduction

Post-translational modifications (PTMs) functionally diversify the proteome [1]. Arginine methylation was first described over 50 years ago. Paik and Kim were investigating methylated products of a calf thymus enzyme (now known as PRMT1) and determined that arginine is a methyl acceptor [2, 3]. The arginine side-chain consists of an aliphatic 3-carbon (C_β, C_γ, C_δ) chain and a terminal guanidinium ion (N_δ, C_ε, $N_\omega/N_{\omega'}$) (Fig. 1a, top). Arginine guanidinium groups remain protonated at physiological pH with a reported $pK_a \sim 12.5–14.0$, the highest of all amino acids [4]. This allows arginine residues to form salt bridge interactions and, as a donor, to participate in up to five hydrogen-bonding interactions. Guanidinium electrons are delocalized into pi-orbitals, allowing for π-stacking interactions. Methylation of arginine does not significantly alter the pKa of the guanidinium functionality [4]. Methylation does alter its shape and charge distribution (Fig. 1b), increases its hydrophobicity, and decreases its H-bonding potential by one per methylation event (for review of methylarginine chemical biology, see [5]). Overall, arginine is a key amino acid regulator of protein–protein and protein–nucleic acid interactions.

The family of protein arginine methyltransferase (PRMT) enzymes catalyzes methylarginine, which occurs on approximately 0.5% [6] to 1% [7] of cellular peptidyl-arginines. PRMTs catalyze the transfer of a methyl group from S-adenosyl methionine (SAM) to an arginine guanidinium group nitrogen (for review of PRMTs, see [8]). All 9 annotated mammalian PRMTs are capable of monomethylation (Rme1, also annotated as MMA) at either terminal guanidinium nitrogen (N_ω) (Fig. 1a, middle). PRMTs are further classified by the type of dimethylarginine catalyzed: Type I PRMTs (PRMT1-4,6,8) catalyze the addition of a second methyl group to the same guanidino nitrogen as the first, generating asymmetric dimethylarginine (Rme2a, also ADMA) (Fig. 1a, bottom left); Type II PRMTs (PRMT5,9) catalyze symmetric dimethylarginine (Rme2s, SDMA)—one methyl group on N_ω and one on $N_{\omega'}$ (Fig. 1a, bottom right); Type III PRMT7 activity is limited to Rme1. In yeast, a Type IV PRMT activity has been described that catalyzes methylation at the internal guanidinium N_ε position (Rme1δ, not reviewed here) [9, 10]. Although Rme1 has long been

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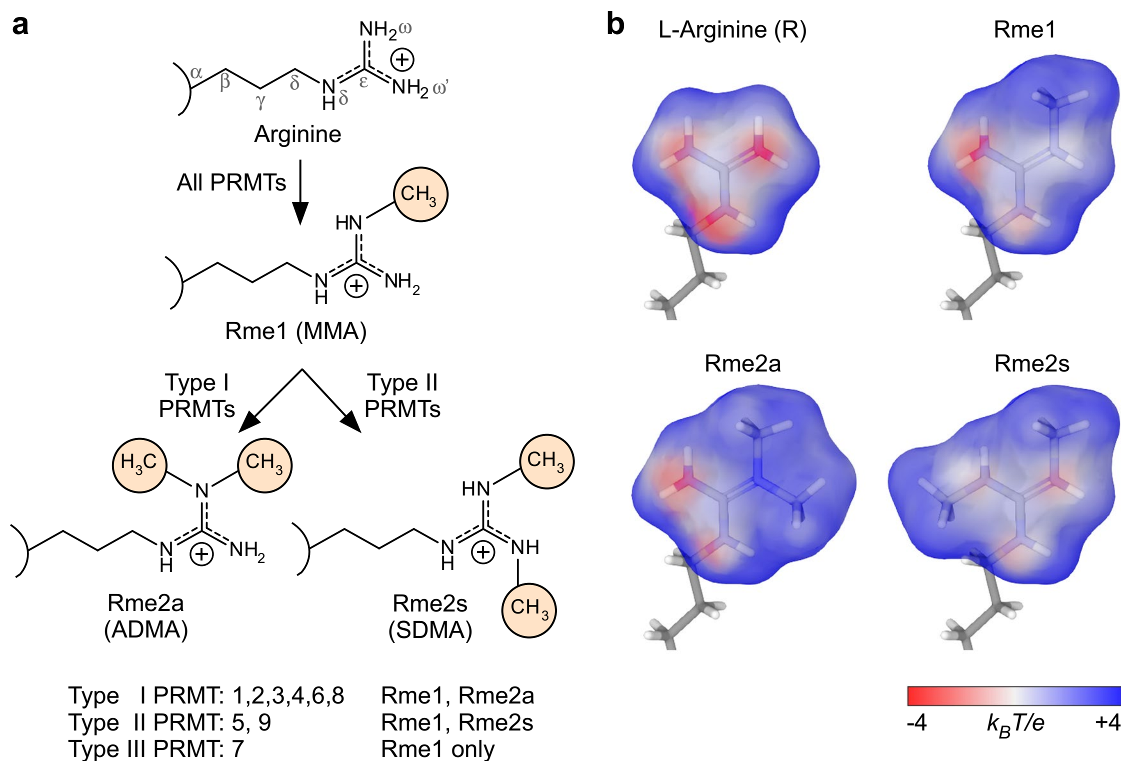

Fig. 1 Arginine methylations and their chemical states. **a** All PRMTs catalyze the addition of one methyl group to one of the terminal ω nitrogens of the guanidinium side chain, producing monomethylarginine (Rme1 or MMA). Type I enzymes (PRMT1,2,3,4,6,8) catalyze a second methylation to the same ω nitrogen, producing asymmetric dimethylarginine (Rme2a or ADMA). Type II enzymes (PRMT5, 9) catalyze a second methylation to the ω' nitrogen, producing symmetric dimethylarginine (Rme2s or SDMA). **b** Electrostatic potential maps of L-arginine and its methylated derivatives reveal a diffuse, positive characteristic. Red = electron dense, blue = electron poor

considered a short-lived intermediate species on the pathway to Rme2a or Rme2s, recent proteome-wide analyses show that Rme1 is abundant, occurring on over 3000 proteins [11, 12]. PRMT1 is by far the most abundant enzyme, responsible for up to 85% of all PRMT activity [13]; PRMT5 is the primary Type II methyltransferase [14]. An important insight for this review is that different PRMT types can share identical substrates. Opposing Rme2a or Rme2s marks could, therefore, allow tuning of biophysical processes. In support of this hypothesis, loss of one PRMT type will often result in "scavenging" by opposing PRMTs. For example, loss of the type I methyltransferase PRMT1 resulted in methylarginine-product switching with a global increase of Type II-catalyzed Rme1 and Rme2s [6]. Although still uncharacterized, dysregulation of PRMTs resulting in these scavenging methylation events likely promotes anomalous cellular physiology and disease.

Arginine methylation occurs on a variety of protein sequence motifs (Table 1). Glycine-and-arginine-rich (GAR) motifs (also referred to as RGG boxes and RGG/RG motifs) are the most commonly reported. Similar motifs with arginine positioned next to an amino acid bearing a small R-group side-chain, such as alanine or serine, are also methylated. Another recurrent PRMT substrate is the RXR motif: two arginine residues separated by any amino acid. Outside of these common motifs, arginine methylation is found at divergent sequences [15, 16], such as AKTRSS (histone H2AR17), VLRDNI (H4R23), and SVYRQQ (Mediator subunit MED12 R206). Furthermore, methylarginine occurs in protein domains of low structural complex (LC) and/or in regions enriched with positively charged residues—features of intrinsically disordered regions (IDRs; Fig. 2) [17]. Indeed, the phenomenon of biological liquid–liquid phase separation (LLPS)—of widespread interest—is frequently mediated through LC domains and charge-enriched IDRs [18, 19].

Effectors of methylarginine marks are largely composed of epigenetic 'reader' proteins and RNA-binding proteins (RNABPs; Fig. 3). These effectors are often subunit components of protein complexes that function in chromatin-templated processes and RNA biology. Only a handful of structural domains that interact with methylarginine marks have been characterized. These include: Tudor (including extended-Tudor (eTUD), tandem tudor (TTD), and other Tudor-like domains; Fig. 4a, b); Trp-Asp 40 repeat (WD40; Fig. 4c); and both plant homeodomain (PHD; Fig. 4d, e)

Table 1 Survey of methylarginine containing proteins

Protein	UniProtKB	Motifs	Arginine # [PRMT]	References (Arg #)
Histones				
H2A	P0C0S8	SGRGK KARAK, VGRVH AERVG, KTRII AIRND	3 [5, 6, 7] 11, 29 [1, 6] 42, 77 88	[224], [225], [226] (3) [227] (11, 29) [228] (42, 88) [229] (77)
H2B	P10853	KKRKRTRKE ASRLA; AVRLL	29, 31, 33 [7] 79, 99	[230] (29,31,33) [228] (79, 99)
H3	Q71DI3	ARTKQTARKS APRKQ AARKS RYRPG EIRRYQ LIRKL, LARRI	2 [5, 6]; 8 [2, 5] 17 [4] 26 [4] 42 [4, 6] 52, 53 63, 128	[225] (5,6); [231], [232] (8) [233] (17) [234] (26) [235] (42) [229] (52, 53) [228] (63, 128)
H4	P62805	SGRGK AKRHRKV VLRDN AIRRL, ETRGV, VIRDA LKRQG	3 [1, 5, 6, 7] 17, 19 [7] 23; 35, 55, 67 92	[236] [224], [94], [226], (3) [230] (17,19) [237] (23) [228] (35,55,67) [229] (92)
DDR/Transcription				
KLF4	O43474	PKRGRRSW	417, 419, 420 [5]	[34]
RUVBL1	Q9Y265	QGRCD	205 [5]	[47]
TDP1	Q9NUW8	PGRFQ; KDRPW	361, 586 [5]	[55]
TOP3B	O95985	QGRGRGR	833, 835 [1, 3, 6]	[60]
RNAP2	P24928	EPRSP; SPRYT	1603 [5]; 1810 [4, 5]	[79], [78]
MED12	Q93074	PVRLP; PTRHL; RLRQQ	1862; 1899; 1912 [4]	[88]; [89]; [90]
TP53	P04637	QIRGRERFE	333; 335; 337 [5]	[238]
MRE11	P49959	GAR motifs, multiple sites	570-665 [1]	[43]
BRCA1	P38398	Unknown sites	504-696 [1]	[45]
mRNP Biology				
SmB	P14678	IGRAAGRGI; QGRGT; PGRGG; MGRGA; PGRGT	108, 112; 147; 172; 181, 209 [5]	[12]; [143]
SmD1	P62314	AGRGRGRGRGRGRGRGRGRGG	98-114 [5]	[142]; [239]
SmD3	P62318	AARGRGRGMGRGN	110; 112; 114; 118 [5]	[142]; [239]
FUS	P35637	GAR motifs, multiple sites	213-218; 242-259; 377-503 [1]	[12]; [161]; [147]; [160]
EWS	Q01844	GAR motifs, multiple sites	300-333; 455-506; 563-638	[12], [240]
TAF15	Q92804	GAR motifs, mainly GDRGG	206 [1]; 431, 459, 475; 483* 528 [1], 535 [1], 562, 570 [1]	[149] (206, 528, 535, 570); [12]
hnRNP A1	P09651	SQRGR FGRGG; SGRGG GSRGG; GGRSS; SGRRF	194 [1] 218 [5], 225 [5] 232, 336, 370	[171] (194) [172] (218,225) [12]
hnRNP A2/B1	P22626	SLRNY; SGRGG; DSRGG; NFRGG; SGRGF; GGRGG; GSRNM; GGRSR	203, 213, 228, 238, 266, 325, 350	[12]
hnRNP A3	P51991	SLREH; RSRGF; SQRGR; RGRGG; MGRGG; FGRGG; GGRGG; GSRGS; SSRGG; GGRSS	52*, 76, 214, 216, 226, 239, 246, 257, 286, 354	[12], [150]
AUF1 (hnRNP D)	Q14103	GSRGG; AGRARGRGG; SRRGG	272, 278, 280, 282, 345	[12]
RBMX (hnRNP G)	P38159	PSRGG; SSRGP; PKRSA; PVRSS	125, 144, 164, 172	[12]
hnRNP H1	P31943	MRRGA	233	[12]
hnRNP K	P61978	PPRGG; GGRGS	316, 377	[12]
DDX4	Q9NQI0	SKRGG; YRRGG; GCRGG; SERGG;	130; 146, 147; 157; 208 [1]	[17]
Lsm4	Q9Y4Z0	KGRGRGG; KGRGM; AGRGV; GGR GRGG; TGRGQ	88, 90; 102; 109 115, 117; 125 [5]	[143]
G3BP1	Q13283	RLRGPGGPRGG; GMRGPPRGG; VGRGL	429, 435; 443 [1, 5]; 447 [1] 460 [5]	[197]

Table 1 (continued)

Protein	UniProtKB	Motifs	Arginine # [PRMT]	References (Arg #)
Immunology				
FOXP3	Q9BZS1	QGRDLRGG	48, 51 [1]	[202]
Signaling				
SMAD4	Q13485	GSRTA	272 [1]	[241]
SMAD6	O43541	GQRGAQGAGRRR	74, 81 [1]	[242]
SMAD7	O15105	PGRAG; AVRGA	57; 67 [1]	[243]
Other				
COBL	Q5NBX1	SERSA; AIRGH	1226; 1234 [2]	[204]
GAPDH	P04406	AFRVP	234 [4]	[209]
SIRT7	Q9NRC8	FGRGC	388 [6]	[211]
ASK1	Q99683	ATRGRGS	78, 80 [1]	[244]

*annotated in uniprot database

and ubiquitin E3 ligase n-recognin (UBR; Fig. 4f) type zinc fingers. Tudor and WD40 domains bind methylarginine marks through cation-π and π–π stacking interactions. Tudor domains use conserved aromatic residues to build an 'aromatic cage' around the interacting guanidinium group (Fig. 4a, b). Similarly, WD repeat protein 5 (WDR5) forms a 'phenylalanine clamp' by stacking the arginine-guanidino moiety between two phenylalanine residues deep within a central cavity (Fig. 4c). Both binding modes are complemented by strong hydrophobic effects generated from desolvation of the charged guanidinium group. While the zinc finger folds are structurally similar, some differences are apparent. The arginine interaction site on the recombination activating 2 (RAG2, Fig. 4d) PHD domain is broad, shallow, and neutrally charged. Whereas the PHD and UBR domains of Ubiquitin-like containing PHD and RING finger domains 1 (UHRF1, Fig. 4e) and UBR1 (Fig. 4f) proteins both present recessed, negatively charged grooves to accommodate arginine binding.

Arginine methylation has long been considered a "permanent" PTM, hypothesized to be removed by enzymatic cleavage or more drastic measures such as histone eviction and/or histone-tail clipping [20]. Protein arginine deiminases (PADs) catalyze hydrolysis of peptidyl-arginine-guanidino group ketimines (=NH_2) into peptidyl-citrulline ketones (=O), releasing ammonia (NH_3) [21]. The charge and functionality of the guanidinium group are lost in the conversion to citrulline which cannot be methylated. A subset of Jumonji domain-containing (JmjC) lysine demethylase enzymes are now known to act on methylarginine residues, catalyzing arginine demethylation via oxidation of the methyl group followed by release of formaldehyde ($H_2C{=}O$) [22, 23]. JmjCs preserve the guanidino functionality, demonstrating that arginine methylation is in fact a reversible PTM. Additionally, two JmjC isoforms 'remove' methylarginine marks by proteolytic clipping of histone H3 and H4 N-terminal tails. Clipping occurs C-terminal to PRMT5-deposited H3R2me2s and H4R3me2s [24].

Here, we review our current understanding of eukaryotic cellular consequences of arginine methylation. We present evidence for how arginine methylation regulates biological processes through modulation of the following three themes: (1) protein PTM crosstalk, (2) functional properties of IDRs, and (3) phase separation of non-membranous organelles.

Arginine methylation and chromatin

Arginine methylation has largely been studied for its role as a histone PTM, where it directly influences reader protein interactions by either facilitating or impairing binding (reviewed [25, 26]). Histones are the major protein component of chromatin—the physiological form of the eukaryotic genome. The combinatorial and reversible nature of histone modifications allow for dynamic modulation of chromatin-templated biochemistries via crosstalk between histone PTMs [27–29]. These crosstalks modulate interactions with epigenetic reader proteins, which are often subunits of multiprotein chromatin-remodelling complexes [30]. Arginine methylation is also prevalent on a host of non-histone proteins involved in numerous chromatin-associated processes [31]. In this section, we discuss how arginine methylation of histone and non-histone proteins influence chromatin-templated processes.

DNA damage response, repair, and genome integrity

Arginine methylation is a central player in the DNA damage response (DDR) system (reviewed in [32]). As mammalian cells sustain much DNA damage due to normal cellular metabolism, replication errors, and environmental factors,

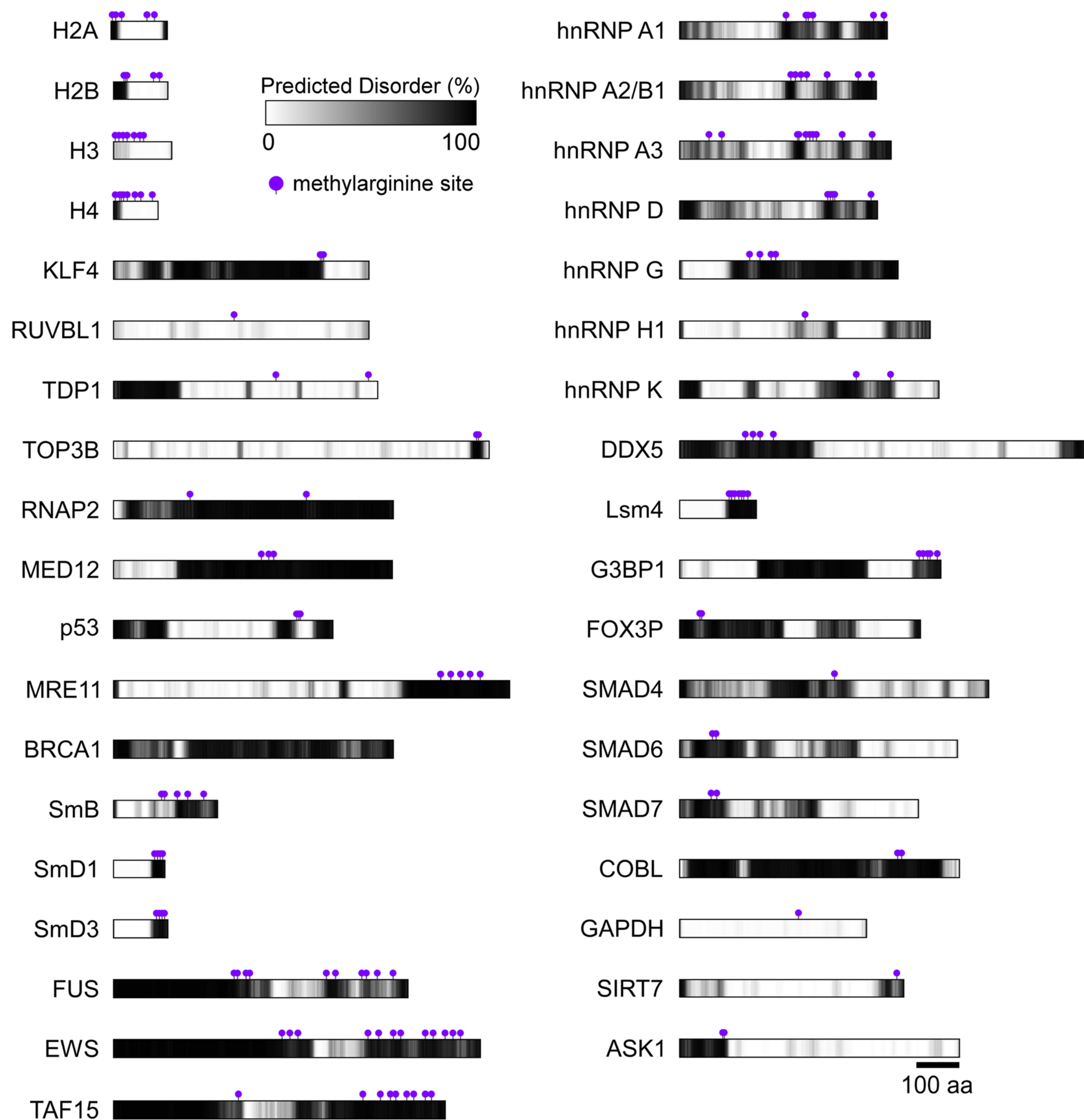

Fig. 2 Arginine methylations are frequently found on protein intrinsically disordered regions. All arginine methylated proteins discussed in this review were analyzed with DISOPRED3. Predicted disordered regions are shown in grayscale on a per-amino acid basis (white = no predicted disorder, black = 100% predicted disorder). Known sites of arginine methylation are indicated with a purple flag. All proteins are full-length except MED12 (1618–2176 aa); RNAP2 (C-terminal 500aa); ASK1 (N-terminal 500aa); BRCA (400–900 aa); and COBL (C-terminal 500aa) All sequences shown to scale, as indicated at the bottom right

the DDR is an elaborate network of molecular systems that detects, diagnoses and, if possible, coordinates the repair of damaged DNA. Arginine methylation has a pivotal role in preserving genomic integrity by orchestrating the elaborate DDR pathways.

Arginine methylation of transcription factor KLF4 (Krüppel-like factor 4) is principal to the decision to pursue DNA damage repair and survival [33]. KLF4 turnover is a key determinant influencing its role in DDR [34]. KLF4 is a short-lived protein ($t_{1/2}$ = ~ 4 h); proteasomal degradation of KLF4 is mediated by the E3 ubiquitin ligase VHL (von Hippel-Lindau), ubiquitylating lysine residues in the KLF4 N-terminal activation domain [35]. PRMT5-dependent methylation of KLF4 inhibits VHL-mediated ubiquitylation, resulting in reduced KLF4 turnover and upregulated p21 that prompts cell cycle arrest and inhibition of apoptosis [34]. Arginine methylation of KLF4 occurs at residues R374, R376, and R377 in a disordered region of the C-terminus (Fig. 2) not captured by structural studies [36, 37]. This suggests that methylarginine deposition may conformationally reorient or occlude the N-terminal region of KLF4 in a manner that antagonizes ubiquitylation but preserves its

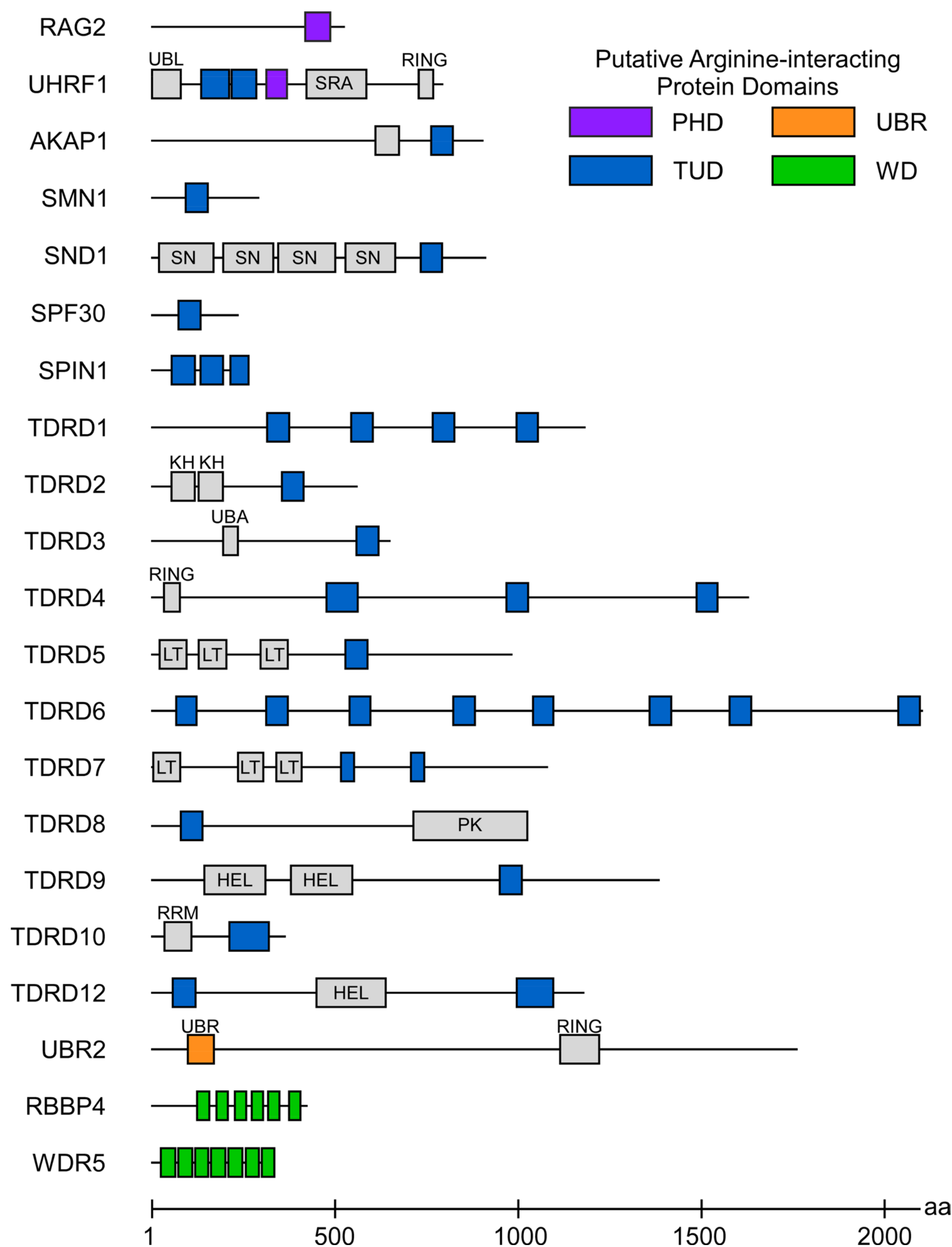

Fig. 3 Selection of proteins with putative arginine-interacting domains. Proteins known to specifically bind to arginine are shown. Domains characterized to interact with arginine are colored as indicated; accessory domains are shown in gray. Helicase (HEL); K homology (KH); LOTUS (LT); (PK); Plant homeodomain (PHD) in purple; (RING); RNA-recognition motif (RRM); Staphylococcal nuclease (SN); SET and Ring-finger associated (SRA); Tudor and tudor-like (TUD) in blue; Ubiquitin-associated (UBA); Ubiquitin-like (UBL); Ubiquitin E3 ligase n-recognin (UBR) in orange; WD40 repeat (WD) in green. All sequences are shown to scale, as indicated at the bottom

transcriptional activity. Nevertheless, upon DNA damage, PRMT5 methylates KLF4 and downregulates KLF4 turnover to promote DNA repair, highlighting a ubiquitin-arginine methylation crosstalk in DDR activation. KLF4 targets many genes and is increasingly investigated for its role in cancer [33]. PRMT5 [14, 38] and KLF4 [34, 39] upregulation are commonly observed in a number of cancers, potentially identifying methylarginine-dependent KLF4 stabilization as a key component promoting carcinogenesis.

Non-homologous end-joining (NHEJ) and homologous recombination (HR) are the main pathways responsible for double-strand DNA break (DSB) repair [40]. PRMT1-catalyzed Rme2a was found on proteins crucial for both types of DSB repair, namely, p53 binding protein 1 (53BP1), meiotic recombination 11 (MRE11), and breast cancer type 1 susceptibility protein (BRCA1) (reviewed [41]). Methylation within the GAR motif of 53BP1 (Fig. 2) is required for its DNA-binding activity [42]; this prevents exonuclease processing by MRE11 at DSBs, preserving dsDNA and stimulating NHEJ repair. Methylation within the GAR motif of MRE11 (Fig. 2) is necessary for nuclease activity and resection at DSBs, exposing single-stranded DNA (ssDNA) and initiating HR-dependent repair [43, 44]. BRCA1 is heavily methylated on the region between 504 and 802 aa (Fig. 2) that contains 12 arginine residues within RXR motifs. Arginine methylation differentially affects BRCA1 recruitment

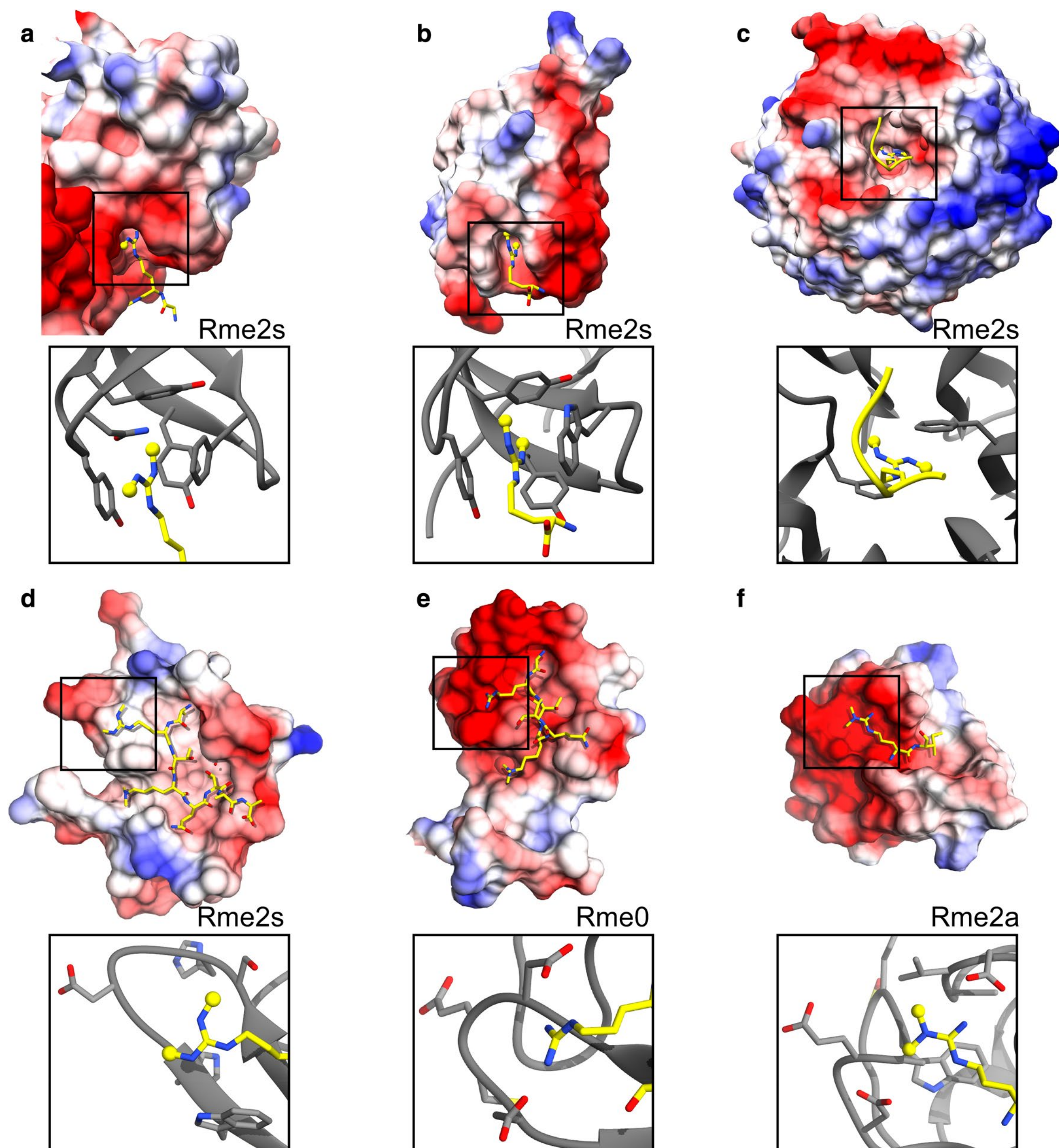

Fig. 4 Representative examples of characterized arginine-interacting domains. Coloumbic potential surface maps are depicted with interacting ligands. Black squares indicate arginine residues. Binding site details are shown in zoomed boxes. **a** Tudor domain, SND1 (anti-syn me2s conformation, PDB: 3OMC) **b** Tudor domain, SMN (syn-syn me2s conformation, PDB: 4A4E). **c** WD40 Repeat domain, WDR5 (PDB: 4A7J). **d** RAG2-PHD (PDB: 2V88). **e** UHRF1-PHD (PDB:3SOW). **f** UBR1 (PDB:5TDB)

to various promoters [45]; however, the effects of Rme2a on BRCA1 function are not well understood. Intriguingly, the methylated region nearly encompasses a BRCA1 DNA-binding domain (498–663 aa) [45], leading to the hypothesis that arginine methylation alters BRCA1/DNA interaction and regulates its tumor suppressor function in DNA repair pathways.

Resistant to ultraviolet B-like protein 1 (RUVBL1) RUVBL1 is a coactivator of TAT-interactive protein 60-kDa complex (TIP60) that catalyzes H4 lysine 16 acetylation (H4K16ac). H4K16ac inhibits 53BP1 from binding DSBs, thereby committing cells to HR-dependent repair [46, 47]. PRMT5-dependent Rme2s plays a key role in directing the NHEJ-to-HR repair type switch during S/G2 of the cell cycle [47]. RUVBL1 and PRMT5 interact in HEK293T and HeLa cells; however, attempts to provide direct evidence of RUVBL1 methylation by PRMT5 using in vitro assays were unsuccessful [47]. Mass spectrometry (MS) analysis of RUVBL1 revealed R205me2s in domain II (DII) of the protein [47, 48]—a domain hypothesized to function in

its biological oligomerization [49]. Although RUVBL1 is not predicted to be disordered (Fig. 2), structural studies show that R205 is located on a flexible region of the protein [48]. Depletion of PRMT5 in HeLa cells subsequently irradiated to induce DNA damage resulted in persistent foci of NHEJ repair markers 53BP1 and γH2AX. Similarly, upon irradiation of RUVBL1-depleted cells, persistent foci were observed, which were efficiently cleared by ectopic expression of RUVBL1^{WT} but not methylation-incompetent RUVBL^{K} (R205K) [47]. Upon DNA damage in PRMT5-deficient cells and in RUVBL1 knockdown cells reconstituted with RUVBL1^{K}, 53BP1 levels increased while H4K16ac decreased [47]. Therefore, activation of HR-dependent DNA repair implicitly relies on RUVBL1 R205me2s catalyzed by PRMT5; this methylation is required for TIP60 acetyltransferase activity, installment of H4K16ac, and inhibition of 53BP1 accumulation at DSBs.

Topoisomerase I (TOP1) relieves superhelical tension resulting from supercoiled DNA created during transcription and replication. After relaxation, TOP1 aligns nicked DNA strands for ligation to restore dsDNA [50]. Failures in ligation result in TOP1 cleavage complexes (TOP1cc) remaining covalently bound to DNA [50]. Replication and/or transcriptional machineries collide with TOP1cc, generating DSBs and consequential cell death [51]. Tyrosyl-DNA phosphodiesterase 1 (TDP1) repairs DNA by excising TOP1cc lesions [52], and genetic disruption of TDP1 presages hypersensitivity to anti-cancer drugs like camptothecin (CPT), which stabilize TOP1cc [53, 54]. PRMT5 methylates TDP1 at R361 and R586, both of which reside in structured non-catalytic regions of the protein (Fig. 2) [55]. MS analysis of TDP1 isolated from control and CPT-treated mouse embryonic fibroblasts (MEFs) revealed that R361me2s was present only in CPT-treated cells, whereas R586me2s was present in both conditions. CPT-induced DNA damage increased TDP1 methylation by ~ 40%. Comparing TDP1-knockout MEF cellular extracts complemented with either PRMT5-treated TDP1^{WT} or TDP1 ^{KK} (R361K/R586K, Rme-incompetent) showed decreased TDP1^{KK} hydrolysis in an ex vivo activity assay. In CPT-treated human colorectal cancer cells (HCT116), immunofluorescence demonstrated a time-dependent increase in repair foci; however, foci were noticeably reduced in PRMT5-deficient cells. Additionally, in CPT-treated TDP1-deficient MEFs and in those expressing TDP1^{KK}, a marked increase of TOP1-associated DNA double-strand breaks were observed compared to cells expressing TDP1^{WT} [55]. These results depict an implicit role for TDP1 arginine methylation in repair of TOP1-associated DNA damage.

Topoisomerase 3B (TOP3B) is the only mammalian topoisomerase that possesses activity toward DNA and RNA, facilitating transcription and translation, respectively [56]. In both the nucleus and cytoplasm, TOP3B associates with Tudor domain-containing protein 3 (TDRD3)—an ssDNA- and RNA-BP and reader of histone H4R3me2a. TDRD3 localizes TOP3B to chromatin or polyribosomes and, furthermore, mediates the switch between distributive and processive topoisomerase activities [56, 57]. Chromatin-associated TOP3B relieves negatively supercoiled DNA and resolves R-loops (three-stranded DNA/RNA hybrids, associated with neurodegenerative disorders and DNA damage [58]) generated by RNA polymerase II (RNAP2) transcriptional activity [57]. Polyribosome-associated TOP3B prevents topological tension in RNA that may arise during transcription or protein translation [59]. TOP3B is methylated on residues R833 and R835 in its disordered C-terminal GAR domain (Fig. 2) [60]. PRMTs 1, 3, and 6 methylate TOP3B in vitro. Rme2a was detected on TOP3B immunoprecipitated from HeLa cells, confirming in vivo methylation. To test methylarginine influence on TOP3B activity, wild type and methylation-deficient (R833K/R835K) TOP3B^{KK} were expressed in and purified from HEK cells and then used in an in vitro DNA relaxation assay. TOP3B^{KK} exhibited substantially reduced activity compared to wild type. In addition, TOP3B^{KK} resulted in increased R-loop formation in vitro, and knockdown of TOP3B increased R-loops in vivo—a phenotype rescued by ectopic expression of wild type but not TOP3B^{KK} [60]. Therefore, TOP3B Rme2a enhances topoisomerase activity and prevents R-loop accumulation, thereby preserving genomic integrity during transcription.

Transcriptional regulation and methylarginine crosstalk

Transcriptional regulation is achieved by many processes that are governed by histone PTMs, including coordination of transcription factors (TFs) and chromatin remodelers. Histone PTM crosstalk has emerged as a central player in chromatin physiology and epigenetic regulation of gene expression. Modulation of RNAP2 dynamics also regulates transcriptional output. Together with DNA methylation, these factors control access to and dissemination of the underlying genetic information. In this section, we discuss the regulatory roles of arginine methylation with respect to gene transcription.

DNA methylation (5-methyl cytosine) at promoter CpG (cytosine–phosphate–guanosine) dinucleotides is a hallmark of transcriptional repression. DNA methylation is established by the de novo DNA methyltransferases DNMT3A and DNMT3B, whereas epigenetic inheritance of DNA methylation patterning is predominantly perpetuated by the maintenance DNA methyltransferase DNMT1 [61, 62]. To faithfully propagate DNA methylation during DNA replication, DNMT1 is recruited to hemi-methylated DNA by the multidomain protein UHRF1 [63]. DNMT1 recruitment to

DNA replication forks is mediated through multiple chromatin interactions mediated by UHRF1 (Fig. 3): the SRA (SET- and RING-associated domain) interacts with hemimethylated DNA [63] and the TTD (tandem Tudor domain) interacts with histone H3K9me2/me3 [64]. In addition, the PHD domain interacts with unmodified histone H3R2; conversely, all forms of H3R2 methylation disrupt UHRF1 binding [65]. Overexpression of PRMT6 in mESCs increased H3R2me2a levels and globally decreased DNA methylation; likewise, chromatin-bound UHRF1 was substantially reduced in chromatin immunoprecipitation (ChIP) and nuclear fractionation assays [66]. In MCF7 breast cancer cells, which exhibit elevated PRMT6 expression, depleting or drugging PRMT6 markedly increased global DNA methylation and dramatically reduced H3R2me2a but did not perturb UHRF1 or DNMT1 levels. These experiments demonstrate PRMT6-dependent H3R2me2a inhibits UHRF1-H3 interaction and, consequently, impedes DNMT1 recruitment to chromatin, resulting in hypomethylated DNA—a signature feature of dysregulated transcription. Furthermore, a strong correlation exists between PRMT6 overexpression and DNA hypomethylation in oncogenic transformation [66–68].

The ADD (ATRX-DNMT3-DNMT3L) domain of DNMT3A contains both a GATA-1-like and a PHD-like zinc finger domain. The PHD domain was first reported to target DNMT3A to chromatin by specifically recognizing PRMT5-dependent H4R3me2s [69]. In K562 erythroid cells, loss of H4R3me2s by PRMT5 knockdown impaired recruitment of DNMT3A and diminished DNA methylation. Hypomethylation resulted in aberrant activation of γ-globin expression, implicating arginine methylation as a direct driver of DNA methylation in fetal-to-adult hemoglobin switch [69]. However, subsequent studies reported that DNMT3A is guided to chromatin by PHD domain interaction with unmodified histone H3K4 and is negated by H3K4me2/3; neither study could recapitulate an interaction with H4R3me2s [70, 71]. The crystal structure of DNMT3A bound to an unmodified H3K4 peptide (PDB:3A1B [70]) supports the H3K4-dependent recruitment model and suggests a potential role for H3R2 methylation in DNMT3A recruitment by directing H3K4me status [72]. Lack of structural support for a DNMT3A/H4 interaction does not necessarily rule out a function for H4R3me in direct or indirect DNMT3A recruitment. This literature does, however, highlight the power of combining structural studies with quantitative binding assays to confirm direct interactions and correlate biological studies with in vitro analysis.

PTMs on the RNAP2 carboxy-terminal domain (CTD) coordinate both transcriptional and non-transcriptional processes. Mammalian RNAP2 CTD is highly disordered (Fig. 2) and contains 52 heptapeptide repeats (YSPTSPS, consensus) [73–77]. R1603 and R1810 occur in two non-consensus repeats (#2 and #31) and are methylated by both Type I and Type II PRMTs [78, 79]. R1810me2a is catalyzed by PRMT4 (more commonly referred to as coactivator-associated arginine methyltransferase 1, CARM1) [78]. In in vitro assays using RNAP2 purified from HeLa cells, CARM1 methylated hypophosphorylated RNAP2 but not the hyperphosphorylated form due to pre-existing methylarginine marks. Pre-phosphorylated RNAP2 CTD peptides were not CARM1 substrates. Thus, in vivo, RNAP2 arginine methylation occurs before phosphorylation and transcriptional initiation. CARM1 depletion in MEFs resulted in misexpression of small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). In human hematopoietic Raji cells expressing an R1810A mutant, this misexpression phenocopied, suggesting that CARM1-dependent R1810me2a transcriptionally influences splicing by moderating availability of spliceosomal and nucleolar ribonucleic acid components. Additionally, although TDRD3 knockdown did not influence transcription of snRNA and snoRNA, RNAP2 CTD-peptide binding assays indicated that TDRD3 specifically interacts with R1810me2a [78]. This suggests that, like H4R3me2a, RNAP2 R1810me2a may recruit TDRD3/TOP3B to chromatin and/or assist in its transfer to RNA co-transcriptionally [57].

Pursuing identification of an in vivo interaction between RNAP2 R1810me2a and TDRD3, co-immunoprecipitation (coIP) experiments with RNAP2 subunit D were performed. Unexpectedly, a CTD variant containing R1810me2s was purified [79]. RNAP2 purified from PRMT5-deficient HEK293 cells lacked R1810me2s; furthermore, CTD peptides containing R1603 or R1810 were both methylated by PRMT5 in vitro. Using a candidate interactor peptide binding assay, R1810me2s bound nuclear Survival of Motor Neuron (SMN) protein. IPs confirmed RNAP2-SMN interaction, and both R1810A mutation or PRMT5-depletion disrupted the interaction in HEK cells. SMN interacts with Senataxin, a helicase responsible for R-loop clearance at transcriptional pause sites, facilitating termination and RNAP2 release [79–81]. CTD R1810A mutation or depletion of Senataxin, PRMT5, or SMN all resulted in persistent and accumulated R-loops at the *ACTB* gene termination region [79]. Thus, to prevent R-loop accumulation at transcriptional terminator sites, SMN binds PRMT5-dependent RNAP2 R1810me2s and recruits Senataxin helicase activity to RNAP2 CTD. In contrast to the termination role of R1810me2s, R1810 citrullination is also implicated in transcriptional pause release and elongation control [82]. Citrullination and arginine methylation are mutually exclusive; therefore, the biological consequences of these opposing regulatory events are likely of great significance and important areas of future study. Collectively, these results further suggest that a crosstalk occurs between arginine methylation, citrullination, and serine phosphorylation at the RNAP2 CTD.

Mediator is a megadalton multi-subunit protein complex conserved throughout Eukarya; it binds RNAP2 to loop it with distal DNA. By interpreting regulatory signals broadcast by enhancer-bound transcription factors and activating non-coding RNAs (ncRNA-a), Mediator modulates transcriptional activity [83–86]. Mediator subunit 12 (MED12) is a key structural component of Mediator's transient kinase module [87]. MED12 directly interacts with ncRNA-a [85], and knockdown of MED12 or certain ncRNA-a results in reduced DNA-looping [83]. The C-terminal IDR of MED12 (Fig. 2) is asymmetrically dimethylated by CARM1 at residues R1862, R1912 [88], and R1899 [89, 90]. MED12 is not fully methylated in cells [90] and also interacts with the putative arginine demethylase Jumonji domain containing protein 6 (JMJD6) [91]. Together, these suggest that MED12 cellular functions are tuned by arginine methylation. R1862me2a and R1912me2a sensitized breast cancer cells to chemotherapeutics [88]. R1899me2a was found to be necessary for MED12's capacity to bind RNA, as knockdown of CARM1 inhibited ncRNA-a interaction [90]. Additionally, MED12 R1899me2a peptide interacted strongly with the Tudor domain of TDRD3 in pulldown assays, whereas R1862/R1912me2a was dispensable for this interaction. MED12/RNA interactions were also found to be TDRD3 dependent. As TDRD3 recruits TOP3B to DNA and RNA, MED12 R1899me2a potentially mobilizes topoisomerase activity to enhancers bound by ncRNA-a to facilitate transcription. As Mediator's role is to assemble large ribonucleoprotein complexes, the presence of arginine methylation mediating some of these interactions suggests a very important role for this PTM. Furthermore, Mediator contains many IDRs [92]; as discussed later in this review, these IDRs are hotspots for both arginine methylation and nucleic acid binding and also contribute to the regulation of phase separation.

Histone PTM crosstalk between arginine and lysine pairs

Arginine methylation of intrinsically disordered histone N-terminal tails (Fig. 2 and Table 1) is implicated in crosstalk to other PTMs—such as between arginine and lysine (RK) pairs—and reader recruitment. A prime example of this crosstalk is the methylation status of histone H3R2 and H3K4. H3R2me2s and H3K4me3 often colocalize at active gene promoters; conversely, H3R2me2a and H3K4me3 are mutually exclusive [93]. H3K4 trimethylation is dependent on the status of H3R2 methylation [72]. PRMT5-catalyzed H3R2me2s is read by WDR5—a subunit component of the mixed lineage leukemia (MLL) H3K4 methyltransferase complex—to recruit MLL and establish H3K4me3 at promoters. Conversely, PRMT6-dependent H3R2me2a excludes WDR5 interaction, antagonizing MLL recruitment [72, 94]. Dysregulation of histone PTM crosstalk can have deleterious effects and contribute to disease and cancer pathogenesis. Alterations in TGFß signaling pathways are also observed in numerous cancers [95]. In A549 lung cancer cells, TGFß treatment resulted in PRMT5-upregulation and concomitant H3R2me1/me2s increase; subsequent WDR5/MLL recruitment increased H3K4me3 and elevated expression of epithelial-to-mesenchymal-transition genes [96]. Knockdown or drugging of PRMT5 or WDR5 abrogated this response, and PRMT5 inhibition also impaired the ability of A549 cells to migrate or invade matrigel, phenotypic traits of invasive cancer [96]. These results show how arginine methylation is central to controlling inducible gene transcription. Furthermore, the cellular consequences determined by opposing H3R2 dimethylarginine marks on H3K4 methylation elegantly highlight the crosstalk between this RK pair.

Rag2 recombinase simultaneously interacts with both H3R2me2s and H3K4me3 [97]. The RAG2 PHD finger forms a groove with which it binds the first six residues of the H3 N-terminal tail (ARTKQT) (Fig. 4d). Quantitative binding studies revealed that RAG2 prefers H3R2me2sK4me3. This was surprising as H3R2me2s decreased H3 tail binding affinities of other PHD finger proteins known to interact with H3K4me3, e.g., UHRF1. This is a striking example of histone PTM crosstalk and, furthermore, demonstrates how methylarginine effectors, even within the same protein-fold family, have evolved to specifically recognize differentially patterned methylation at RK pairs.

H3K4/H3R8 constitutes another RK pair engaging in histone PTM crosstalk. The reader protein Spindlin-1 (SPIN1) is a transcription factor implicated in rRNA expression and Wnt-signaling. SPIN1 also has increasingly emergent roles in cancer [98–100]. SPIN1 has three Spin/Ssty2 Tudor-like domains that interact with H3K4/H3R8. [101–103]. SPIN1 co-crystallized with an H3R8mes2a/H3K4me3 peptide (PDB: 4MZF). Strikingly, SPIN1 affinity for H3K4me3-containing peptides progressively increased with modulation of H3R8 methylation states (H3R8me0 < me1 < me2s < me2a) [102]. Furthermore, ChIP studies revealed overlap of R8me2a and K4me3 at common Wnt-responsive promoters [102]. Together, these results indicate that SPIN1 recruitment to chromatin results from synergistic crosstalk between H3K4me3 and H3R8 methylation.

H4K20me3 and H4R23me2a form another SPIN1-interacting RK pair, potentially engaged in antagonistic crosstalk [103]. An X-ray crystal structure of SPIN1 complexed with an H4K20me3 peptide was solved (PDB: 5Y5W). In vitro, SPIN1 did not bind R23me0 but did bind H4R23 peptides with greater affinity as methylation progressed from me1 to me2s to me2a. However, an H4K20me3R23me2a peptide bound SPIN1 with ~ 8-fold less affinity than H4K20me3 but with ~ 5-fold greater affinity than H4R23me2a, suggesting that, when dually methylated, simultaneous recognition of

this RK pair is unlikely and may function to temper SPIN1 interactions with the H4 tail [103]. Intriguingly, four residues separate H3K4 and H3R8, whereas three residues separate H4K20 and H4R23, and two residues separate H3R2 and H3K4. This linear separation suggests a potential physical mechanism of reader recruitment by PTM crosstalk. We speculate that other bivalent RK methylation pairs may exist with appropriately spaced methylation marks.

Histone PTM crosstalk also occurs across different histone types. PRMT5-catalyzed H3R8me2s and H4R3me2s are associated with gene repression in development and differentiation (reviewed in [14]). In basal epidermal keratinocytes, H3R8me2s and H4R3me2s colocalize and antagonize expression of involucrin (IVL)—a transcriptional activator required for differentiation. Furthermore, stimulation of the PKCδ/p38δ pathway increased IVL expression and decreased PRMT5 and H3R8me2s/H4R3me2s levels at IVL promoter (hINV), demonstrating how regulation of arginine methylation functions in keratinocyte differentiation [104]. Recall that KLF4 half-life is increased when methylated by PRMT5 during DDR signaling [34]. KLF4 is also important for keratinocyte differentiation, and KLF4 expression is stimulated by PKCδ; however, its half-life remained unaffected [105], consistent with the hypothesis that PKCδ inhibits PRMT5 expression. This is also consistent with the idea that shorter-lived signaling pathways, such as differentiation of proliferating cells, are expedited by quick turnover of responsive proteins; in contrast, in longer-lived pathways, such as cell cycle arrest, reduced turnover rate of responsive proteins is necessary. KLF4 methylation provides a prime example of how arginine methylation of a single protein regulates transcriptional activities in diverse cellular processes.

It has not escaped our notice that crosstalk between additional histone RK pairs exists, such as H3R17/H3K18, H3R26/H3K27, and H4R3/H4K5 (for review of previously identified histone arginine/lysine interplays, see [106]). For example, CARM1 methylates H3R17 when H3K18 is acetylated but not when H3K18 is unmodified [107]. Likewise, CARM1 preferably methylates H3R26 when H3K27 is acetylated but not when H3K27 is trimethylated [108]. Therefore, CARM1 activity is stimulated on substrate arginines by prior acetylation of adjacent lysine residues (neutral charge), whereas unmodified and methylated lysine residues (positive charge) deter CARM1-dependent methyltransferase activity [106]. In a reciprocal fashion, PRMT1-dependent H4R3me2a stimulates p300 acetylation [109] of H4K8 and H4K12 [110], whereas prior acylations (acetylation, butyrylation, crotonylation) at H4K5 prevent PRMT1 activity at H4R3 [111]. Thus, it is becoming increasingly clear that crosstalk between histone arginine methylation and histone lysine modifications functions collectively to regulate chromatin-based processes and downstream cellular consequences. Future interrogation of additional modifications such as citrullination, phosphorylation, and ubiquitylation will likely expand our understanding of histone PTM interplays.

Arginine methylation in ribonucleoprotein biology

Messenger RNA (mRNA) transcripts are dynamically bound by > 1000 proteins, forming messenger ribonucleoprotein particles (mRNPs)—the physiological form of the transcriptome (reviewed [112]). Arginine methylation is present on many RNABPs [113] and contributes to regulating nearly every aspect of mRNP biology, from nascent pre-mRNA stabilization and splicing, through nuclear export, to cytoplasmic localization, during translation, and into decay (previously reviewed [113, 114]). RNABP IDRs are increasingly reported to drive compartmentalization and efficiency of cellular processes by organizing non-membranous phase-separated organelles (PSOs, reviewed [115]) via formation of reversible LLPS droplets, hydrogels, and fibrillary gels ([116, 117], reviewed [118, 119]).

Chromatin is perhaps the largest PSO within the nucleus [120]. Other PSOs—such as nucleoli, Cajal bodies, and speckles—participate in rRNA and ribosome biogenesis, snRNP assembly, and as storage wells for a variety of splicing factors, respectively [121]. As a means of regulating their function, mRNPs undergo dynamic reorganization and reversibly form PSOs, such as germ granules, mRNA transport granules, processing bodies (P-bodies), and stress granules (SGs). Germ granules function in early embryogenesis and contain maternal proteins and mRNA transcripts that are consumed prior to transcriptional activation of the embryonic genome (reviewed [122]). P-bodies function in mRNA turnover and contain de-adenylated transcripts and components of the mRNA decay machinery, e.g., decapping enzymes, and Lsm1-7 proteins (reviewed [123]). SGs are conglomerates of non-translating mRNAs and proteins, such as translation factors, kinases, RNA helicases, and signaling molecules that form when cells experience stress (e.g., heat shock, oxidation), resulting in stalled or failed translation initiation (reviewed [124]). Arginine methylation status of RNABPs is linked to both normal physiological LLPS and dysregulated pathological accumulation of irreversible filamentous, ß-amyloid-like masses. These disease-associated aggregates are especially prevalent in neurodegenerative proteinopathies such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia/lobe degeneration (FTD/FTLD), multiple sclerosis (MS), Alzheimer's disease (AD), Huntington's disease (HD), as well as in numerous cancers [125–127]. In this section, we discuss arginine methylation as it applies to mRNP biology, phase separation, and associated disease.

snRNPs and spliceosome biogenesis

SMN is responsible for building the Sm ("Smith antigen")-core—a heptameric ring of Sm proteins (B/B', D1, D2, D3, E, F, G)—on U1, U2, U4/U6, and U5 snRNAs to form snRNPs, which are vital for spliceosome assembly [128] (for review of SMN, see [129]). The intrinsically disordered C-terminal tails of SmB/B', SmD1, and SmD3 (Fig. 2) are rich in GAR motifs (Table 1) that are methylated by PRMT5 complexed with methylosome protein 50 (MEP50) and the Ion Chloride nucleotide sensitive protein (pICln) [130]. The SMN Tudor domain (Fig. 3b) binds directly to SmB/B', SmD1, and SmD3 in an arginine methylation-dependent manner, facilitating Sm-ring construction [131]. Deletion of PRMT5 in murine neural progenitor cells results in post-natal death due to aberrant splicing of cell cycle genes, such as MDM4, at weak 5′-donor sites [132]. In PRMT5-depleted hematopoietic cells, decreased global Sm protein dimethylation results in defective splicing and deficient DNA repair, eventuating cell death [133, 134]. Likewise, in primary spermatocytes, TDRD6 interacts with PRMT5 and with arginine-methylated SmB prior to snRNP formation, suggesting that TDRD6 guides Sm proteins to PRMT5 for methylation and subsequent snRNP assembly [135]. In TDRD6-deficient diplotene spermatocytes, loss of SmB dimethylation leads to impaired spliceosome maturation and defective splicing, which ultimately suspended spermiogenesis [135, 136]. Furthermore, as cancer cells are addicted to PRMT5′s function in splicing, development of therapeutics targeting PRMT5 may be of high clinical significance [137].

Auto-antibodies against Sm proteins were discovered over 50 years ago [138] and were subsequently used to isolate snRNPs [139]. As snRNP functions were discovered [140], it was soon discovered that SMN interacts with Sm protein GAR motifs [141]. It became clear that Rme2s played a critical role in assembly of the spliceosome [142, 143]. Interestingly, Rme2s was the actual "Smith" antigen on SmD1/D3 [144]; Rme2a was also reported to occur on Sm protein [145]. Furthermore, X-ray crystal structures of SMN bound to Rme2s and Rme2a were solved [146], suggesting that dynamic Sm methylarginine isoforms are biologically significant.

Non-Sm proteins in splicing, phase separation, and disease

Arginine methylation is prevalent on numerous non-Sm proteins fundamental to mRNP biology, such as these classes: (1) FET proteins (Fused in Sarcoma, also translocated in liposarcoma, FUS/TLS [147]; Ewing sarcoma, EWS [148]; and TATA-associated factor 15, TAF15 [149]); (2) heterogeneous nuclear RNPs (hnRNPs) A1, A2/B1, A3, D, G, H, K, and others [114, 150]; and (3) DEAD-box (DDX) RNA helicases (Fig. 2). Some of these proteins remain bound to RNA and regulate their further processing, nuclear export, cytoplasmic functions, and mRNP phase behavior. These proteins are enriched with LC domains, IDRs, and GAR motifs that directly participate in LLPS. Furthermore, dysregulated phase separation arising from mutation or impairment of arginine methylation within these proteins results in their cytoplasmic accumulation and disease-associated irreversible aggregation [126, 147, 151–153].

FET proteins

FET proteins are predominantly disordered (Fig. 2). Their domain structure comprises an N-terminal transcriptional activation domain of low complexity and a semi-structured C-terminal RNA-binding domain (RBD) followed by a non-canonical proline–tyrosine nuclear localization signal (PY-NLS, interacts with transportin (TRN) nuclear import receptor). The semi-structured RBD contains three disordered GAR motifs interspersed with a structured RNA recognition motif (RRM) and zinc finger domain [154]. Transcriptional activation is primarily observed by FET fusion proteins arising from oncogenic translocation. Wild-type FET transcriptional activity is hypothesized to be auto-inhibited in part by cation-π interactions between GAR motif-arginines and activation domain aromatic residues [155], suggesting that auto-inhibition may also be affected by arginine methylation. Recent attention has been focused on FET proteins, especially FUS, largely due to the potential of their LC domains and IDRs to undergo LLPS. In addition, these proteins are prominent components of PSOs, such as mRNA transport granules and SGs. FET proteins are suggested to accumulate downstream of SG formation, i.e. they are not required for nucleation [127], and likely function to condense and shield RNA transcripts in a reversible manner. Furthermore, arginine methylation, serine/threonine phosphorylation, glycosylation, and ubiquitylation PTMs occur on FET proteins [156–158]. Thus, dynamic PTMs of FET protein IDRs may function to shuttle them in and out of granular assemblies, mediating phase behavior and mRNA accessibility. Further interest in FET proteins is sparked by their association with a variety of diseases, as translocations, mutations, and recently arginine methylation status have been shown to dysregulate LLPS, leading to the formation of anomalous SG-like cytoplasmic aggregates observed in cancers and numerous neurodegenerative disorders [116, 117, 127, 156].

FUS is integral to many roles in mRNA biology during early vertebrate gastrulation and neurogenesis [151, 159]. At least 20 different sites of PRMT1- or PRMT8-catalyzed Rme2a occur in FUS GAR motifs [160, 161]. As arginine methylation occurs in the third GAR motif (RG3, 473-505aa) and within PY-NLS (506-526aa; R514, R518, R521, R522 and/or R524), it is hypothesized to affect FUS subcellular

localization [147]. ALS-associated mutations in PY-NLS (R521G, R524S, R522G) exclude arginine methylation and were also thought to impair FUS localization. To test these hypotheses, HeLa cells expressing wild-type FUS (FUS-WT) and ALS mutants (which mislocalized in the cytoplasm) were treated with the general methylation inhibitor adenosine dialdehyde (AdOx). FUS-WT and, strikingly, all ALS mutants were primarily localized to the nucleus after AdOx treatment. A separate report confirmed these results and showed that PRMT1 depletion in HEK cells and in dissociated murine spinal cord motor neurons also abrogated cytoplasmic mislocalization of ALS mutants [162]. Similar results were observed with EWS- and TAF15-mutants found in FTLD-FUS aggregates [147]. These results suggest that arginine methylation comparably affects nuclear translocation of all FET proteins; however, methylarginine marks within PY-NLS appear to have no significance here. Consistent with this hypothesis, a GFP-reporter assay showed that only GFP-RG3 successfully localized the nucleus, whereas both PY-NLS and Arg-to-Lys mutant RG3 both failed to import into the nucleus [147]. Overexpression of PRMT1 in HEK cells slightly increased cytoplasmic FUS levels in cell fractionation assays, suggesting a role for Rme2a in FUS localization or retention in the cytoplasm [13]. HSQC NMR analysis confirmed that the FUS C-terminus containing RG3 and PY-NLS is intrinsically disordered. Additionally, the loss of glycine peaks observed upon addition of TRN demonstrated that the RG3 region directly interacts with nuclear import receptor [147]. To directly test the hypothesis that FUS Rme2a influences TRN binding, NMR and pulldown assays were employed using unmodified and RG3-Rme2a FUS peptides, which confirmed that Rme2a disrupts TRN interaction [147]. PRMT1 knockout resulted in elevated levels of unmodified and monomethylated FET proteins and loss of Rme2a. Furthermore, unmodified and Rme1-FUS peptides were shown to enhance TRN interaction [163]. These experiments clearly point to a regulatory mechanism for FUS (and likely for EWS and TAT15) subcellular localization by which arginine methylation within FET-RG3 motifs directly influences TRN interaction and nuclear translocation; this process is dysregulated in FET protein-associated disease [147].

A distinguishing feature between FUS-associated FTLD and FUS-ALS inclusions is that TRN is present in the former but not the latter [162]. More than 50 mutations near PY-NLS are associated with FUS-ALS [164]. As these mutations do not influence their Rme2a levels, FUS-ALS mutants fail nuclear import and accumulate in the cytoplasm in part due to their inability to bind TRN—implicating Rme2a as a potential driver of FUS-ALS aggregation. On the other hand, FUS-FTLD arises not due to mutation but rather hypomethylation; thus, inclusions contain unmodified and monomethylated FUS [163]. FUS LLPS is reported to occur as cytoplasmic FUS levels breach threshold and begin accumulating in SGs, eventually undergoing irreversible liquid-to-solid phase transition (LSPT) [117, 165]. In this light, TRN also functions as chaperone to discourage phase separation of hypomethylated FUS in FTLD; conversely, TRN chaperone activity is abrogated by Rme2a, leading to accumulation and condensation of FUS in ALS [116, 117]. FUS likely self-assembles through intermolecular interactions between its N-terminal prion-like LC domains [117, 166]. Arginine methylation was initially reported to not have an effect on FUS solubility [163]. Recently, however, C-terminal RG3-PY domain arginines were found to be critical for regulating FUS phase behavior [116, 117]. PRMT1-dependent Rme2a reduced FUS LLPS, requiring higher concentrations for demixing compared to hypomethylated FUS [117]. This was proposed to result from diminished intermolecular H-bonding between LC and RG3 domains due to arginine methylation, which interferes with cross-ß-sheet structural interactions vital to LLPS [116]. Therefore, GAR motif Rme plays a central part in FET protein biochemistry, directing subcellular localization and chaperoning cytoplasmic phase behavior, by tuning their interactions with TRN. hnRNPs—another class of intrinsically disordered RNABPs that that influence LLPS—are discussed next.

hnRNPs

hnRNPs are core components of mRNPs. Some hnRNPs are transported back to the nucleus after delivering processed transcripts to the cytoplasm, while others remain and regulate cytoplasmic mRNP processes, including incorporation into and out of P-bodies and SGs. [167, 168]. Additionally, hnRNPs are linked to pathogenesis of cancer and numerous neuropathies (for review, see [169]). Early studies into the cellular distribution of arginine methylation reported that ~ 65% of Rme2a in the nucleus occurred on hnRNPs [170]. PRMTs methylate arginines within hnRNP RRMs and GAR motifs—the functions of which we discuss next.

hnRNPA/B protein family members consist of hnRNPs A1, A2/B1, A3, and A0, each of which contains two N-terminal RRMs and a C-terminal IDR containing several GAR motifs and an LC domain (Fig. 2). Arginine methylation of hnRNP A1 GAR motifs occurs by both PRMT1 [171] and PRMT5 activities [172]. Early work on hnRNPs reported that PRMT1-dependent Rme reduced hnRNP A1 RNA- and ssDNA-binding capacity [171]. hnRNP A1 is now known to aid in cellular stress recovery by associating with and resolving SGs [173]; additionally, hnRNP A1 GAR motifs are important for translation at internal ribosome entry sites (IRES), functioning as IRES-transactivating factors (ITAFs) [153]. Using wild-type and mutant hnRNP A1 GAR motifs, i.e., Arg-to-Lys (unmodified arginine mimic) and Arg-to-Ala substitutions, which were engineered for cytoplasmic

retention, Rme was found to be dispensable for RNA binding and SG association. The Arg-to-Ala mutant, but not Arg-to-Lys, failed to associate with RNA in IP experiments and had markedly reduced association with SGs in cellular stress assays. Thus, arginine residues within hnRNP A1 GAR motifs are required for RNA and SG interactions. In IRES translation assays, GAR motif Rme2a was shown to significantly suppress ITAF activity. Additionally, wild type and lysine-substituted GAR motifs both reduced SGs to an equal extent during stress assay recovery periods. These results show that GAR motif arginines are necessary for cytoplasmic functions of hnRNP A1 and that Rme2a decreases ITAF activity [153], potentially to decommission translation in SGs.

PRMT5 also methylates hnRNP A1 GAR motifs at R218 and R225 [172]. Knockdown or drugging of PRMT5 significantly repressed translation of four different IRES reporters; furthermore, two of these IRES were known to be regulated by hnRNP A1. To test Rme2s dependence on IRES activity, native, single-, or double-site (R218K/R225K) hnRNPA1 were expressed in hnRNP A1-depleted HEK cells containing an IRES reporter. Only the double mutant failed to rescue IRES translation deficits created by hnRNPA1 depletion, suggesting that R218 or R225 is necessary for IRES translation. hnRNP A1 Rme2s does not affect its subcellular localization. However, in PRMT5 knockout MEFs, loss of Rme2s significantly reduces hnRNP A1-IRES interaction. This study illustrates a regulatory mechanism for hnRNP ITAF activity being activated by PRMT5-dependent Rme2s and inactivated by PRMT1-dependent Rme2a—again highlighting the interplay between different forms of arginine methylation.

A role for Rme at hnRNP A1 RRM was discovered to function in drug-resistant pancreatic cancer [174]. PRMT3 catalyzes R31me2a in the first RRM of hnRNP A1, increasing its ability to bind and stabilize mRNA transcripts of the ATP-binding cassette subfamily member G2 (ABCG2) drug-efflux protein pump. Overexpression of ectopic PRMT3 in pancreatic cancer cell lines resulted in upregulation of ABCG2. PRMT3 is also upregulated in drug-resistant pancreatic cancers; thus, inhibition of PRMT3 and consequent reduction of hnRNP A1 arginine methylation may prove to be a successful strategy in combatting chemoresistance [174, 175]. hnRNP A1 RRMs are associated with G-quadraplex DNA that function in telomere protection, and hnRNP A1 GAR motifs interact with and enhance G-quadraplex unfolding, promoting telomerase activity [176, 177]. It will be interesting to see how arginine methylation of hnRNP A1 RRMs and GAR motifs influences its function at telomeres.

Splice variants hnRNP A2 and B1 GAR motifs are PRMT1 substrates, modified with both Rme1 and Rme2a [178]. Studies reporting the degree to which Rme occurs vary, ranging from Rme2a at a single site (R254, rat brain) [150] to multiple sites of Rme1 within all four C-terminal GAR motifs (T-cells) [16], suggesting that arginine methylation patterns are cell type specific. PRMT-1 methylation of hnRNP A2 in vitro was restricted to four residues within GAR motifs [126]. hnRNP A2/B1 arginine methylation was first reported to facilitate import into the nucleus, as cytoplasmic localization was increased upon deletion of GAR motifs or AdOx treatment [179]. Conversely, hnRNPA2/B1 Rme was reported to have no influence on nucleo-cytoplasmic distribution—this conclusion was based on an R-to-A mutant of the single R254me2a site that found no difference in subcellular localization [150]. Thus, arginine methylation outside of R254 likely does influence hnRNP A2/B1 nuclear localization, potentially in a cell type-specific manner. As with FET proteins, arginine methylation of hnRNP A1 and A2/B1 also mediates LLPS behavior.

hnRNP LLPS phase behavior is mediated by its LC domain, containing a prion-like domain surrounded by multiple GAR motifs [126]. Increased hnRNP expression or mutations within LC domains often results in dysregulated phase separation and accumulation of cytoplasmic aggregates observed in many pathologies [165, 180]. Splicing factor transactivating response DNA-binding protein 43 kDa (TDP43) is a physiological binding partner of hnRNP A2 and is commonly found together with FUS and hnRNPs in disease-associated inclusions [181]. Using solution NMR, a study probing the mechanism behind hnRNP self-assembly and LLPS found that the hnRNP A2 LC domain is compact and structurally disordered as a monomer. Additionally, hRNP A2 LC remains predominantly disordered when phase separated and participates in weak (Kd ~ mM), broadly distributed, multivalent interactions [126]. An aggregation-prone D290V disease-associated mutant enhanced local intermolecular interactions. This suggested that chemical changes in hnRNP-LC domains mediate self-association and potentially nucleate LLPS, similar to that observed with both hnRNPA1 [165] and FUS [182]. Phase separation of hnRNP A2 LC domain induces co-condensation and aggregation with TDP 43 LC domains. NMR analysis revealed that hnRNPA2–TDP43 interactions were broadly distributed throughout the length of their LC domains. This demonstrated that LC domain interactions contribute to liquid–liquid demixing, by self-associating, and/or by comingling with other protein LC domains prone to undergo LLPS.

To investigate the role of arginine methylation on LLPS, hnRNP A2 LC was methylated by PRMT1 in vitro and brought to critical concentration for phase separation. Compared to the native protein, after centrifugation PRMT1-modified hnRnNP A2 LC remained 50% more concentrated in the supernatant, demonstrating that Rme2a decreases LLPS. Molecular simulations showed that Rme2a LC domains are structurally expanded and that GAR motif-Rme2a disrupts 'interaction hotspots' observed between

aromatic residues and unmodified GAR motif arginines important for LLPS. FUS and hnRNPs are also subject to citrullination; citrullination of FUS and hnRNPs is inversely correlated with ALS-associated aggregation, thus implying a direct role for arginine methylation of IDRs in perturbing phase separation and protein aggregation [183].

Other mRNP proteins in germline, processing body, and stress granules

DDX4 (also known as Vasa, as first identified and described in *Drosophila*) is a putative RNA-helicase enriched in germ granules (also known as nuage (oocytes) and chromatoid bodies (spermatocytes))—a dense, 'cloudy' fibrogranular structure present in germ cells and early embryos instrumental for translation initiation of stored maternal transcripts. DDX4 also has emerging roles in ovarian cancer stemness [184–186]. DDX4 possesses an internal helicase domain that is flanked by intrinsically disordered N- and C-terminal tails (Fig. 2). DDX4 IDRs promote LLPS droplets that favor inclusion of ssDNA but not dsDNA [17]. Arginine methylation of DDX4 and related helicases is conserved from planar worms to humans [17, 187, 188]. PRMT1-dependent methylation of DDX4 N-terminal GAR motifs disrupted LLPS, dramatically reducing the ordered-to-disordered phase transition temperature by ~ 25 °C [17]. Mechanistically, DDX4 N-terminal IDRs mimic block co-polymer architecture, clustering similarly charged residues into ~ 10aa 'blocks' of alternate net charge. Scrambling DDX4 IDR charge blocks without perturbing overall net charge abrogated LLPS. Additionally, FG/GF and RG/GR dipeptides cluster within regions of net positive charge, and mutating DDX4 IDR FG/GF pairs to AG/GA pairs disrupts LLPS. These observations show that both electrostatic and cation-pi interactions are required for phase separation. Furthermore, this suggests that DDX4 GAR motif-Rme discourages phase separation by disrupting quadrupolar cation-pi interactions. Intriguingly, FG/GF pairs are separated by ~ 10aa and RG/GR pairs by 4aa, suggesting a selective pressure for the spacing of these dipeptides within DDX4 IDRs. Analysis of the human proteome identified over 1500 similar sequences; moreover, sequences in the top 10% mapped to known proteins with high LLPS propensity that are present in a variety of PSOs. This reveals a potential Rme2a-mediated LLPS-"fingerprint" for this protein subclass: alternating blocks of opposing net charge, with enrichment of specifically placed FG/GF and RG/GR pairs within regions of net positive charge.

Lsm (Like-Sm) proteins form heptameric ring complexes that encircle pre-snRNAs, pre-mRNAs, and pre-tRNAs. Lsm-rings function in various processing steps from splicing (Lsm2-8) to decay (Lsm1-7) [189]. Lsm1-7 binds deadenylated mRNA and is a conserved component vital for 5′-to-3′ mRNA decay [190]; these are often found in phase-separated P-bodies (reviewed [123]). Human Lsm4 has 8 arginine residues within its disordered C-terminal GAR motif (Fig. 2). While this motif is dispensable for Lsm complex formation, translational repression, and decay, it is required for P-body accumulation [191]. Consistent with this idea, the Lsm4 IDR has a high propensity to form LLPS droplets that quickly progress into amyloid-like fibrils [186]. PRMT5 catalyzes Lsm4 Rme2s, and depletion of PRMT5 extinguishes P-body accumulation. Deletion or mutagenesis of Lsm4 GAR motifs failed (RtoA) to or partially (RtoK) rescued P-body formation in Lsm4-depleted cells. Thus, PRMT5-dependent Rme2s of Lsm4 GAR motifs may directly influence phase behavior to promote P-body amassment [191]. Intriguingly, [G/S]Y[G/S] repeats, prevalent in IDRs, promote mRNP-granule-like hydrogel formation [192], suggesting that 'GRG' repeats, dependent on Rme, may have a similar function [191].

Recall that CARM1 installs Rme2a on TOP3B to stimulate its topoisomerase activity and enhance TDRD3 association [60]. TDRD3 was previously identified as a component in SGs and its Tudor domain was necessary for recruitment [193]. TOP3B colocalizes with TDRD3 in SGs and readily forms large cytoplasmic foci in response to stress [60]. Mutation of TOP3B (R833K/R835K) or Type I PRMT inhibition reduced both the number and size of SGs; however, TDRD3 SG-localization was not disrupted. These results suggest that TOP3B, in an Rme2a-dependent fashion, is recruited to SGs by interacting with TDRD3 Tudor domain.

Ras-GAP SH3-binding protein 1 (G3BP1) is an RNase enzyme important in signal transduction and is a nucleating factor in SG formation. The G3BP1 C-terminus contains an RRM and a disordered GAR motif (Fig. 2). Stimulated by Wnt3a, G3BP1 methylation is catalyzed by PRMT1, which decreased G3BP1 binding to *Ctnnb1* (ß-catenin) mRNA [194]. G3BP1 is also important in assembling SGs to preserve mRNA transcripts [195, 196]. PRMT5 also methylates G3BP1; however, this study showed that demethylation of G3BP1 was a determining factor for its role in seeding SGs [197]. A G3BP1 GAR motif deletion mutant expressed in U2OS G3BP1-knockout cells failed, unlike native G3BP1, to form SGs even under oxidative stress; conversely, overexpression of PRMT1 or PRMT5 resulted in reduction of SGs, even when oxidatively stressed. Knockout or inhibition of either enzyme increased SG formation. In addition, R447 and R460 were shown to be methylated by PRMT1 and PRMT5, respectively; R429, R435, and R443 were differentially methylated by either enzyme. Immunoblots revealed that G3BP1 was demethylated under oxidative stress and re-methylated during recovery. MS analysis of G3BP1 isolated from stressed cells confirmed that GAR motifs are demethylated. These results demonstrate that both G3BP1 GAR motif Rme2a and Rme2s suppress SG formation; moreover, they establish that demethylation promotes G3BP1-dependent SG

nucleation. Intriguingly, different stressors produced different Rme responses: ER stress and heat shock both reduced Rme2s, whereas both Rme2a and Rme2s are reduced during oxidative stress. Nonetheless, demethylation appears to be global mechanism triggering G3BP1-dependent reversible LLPS and SG stability.

G3BP1 demethylation was linked to JMJD6 activity [198]. JMJD6, which is predominantly nuclear, localized in cytoplasmic foci coincident with G3BP1 and other SG markers upon subjection to the stressors mentioned above, identifying JMJD6 as a novel SG component. Overexpression of JMJD6 reduced global Rme2a but did not affect Rme2s, indicating that JMJD6 demethylation is Rme2a specific. JMJD6 depletion, inhibition, or expression of catalytically dead mutant impaired SG formation with no effect on global Rme2a. MS analysis of G3BP1 methylation in oxidatively stressed cells overexpressing JMJD6 showed decreases in both Rme1 and Rme2a; JMJD6 knockdown did not significantly affect methylation. Furthermore, in rescue experiments using JMJD6-depleted cells under oxidative stress, expression of mutant JMJD6 failed to rescue SG assembly, whereas native JMJD6 increased SG accumulation. These experiments provide an eloquent example of how arginine methylation can be utilized in a reversible manner to regulate cellular physiology.

These studies provide prime examples of how arginine methylation in IDRs functions in mRNP biology, mediating several biophysical processes from protein-nucleic acid interactions to LLPS. Next, we survey a broad range of human physiology influenced by arginine methylation.

Arginine methylation of other cellular proteins

T-cell and B-cell differentiation and maintenance

Maintenance of cluster of differentiation 4 positive (CD4+) T-helper, CD8+cytotoxic, and invariant natural killer (iNK) T cells is dependent on PRMT5 activity, revealing a regulatory role for arginine methylation in γc-dependent Jak3-signaling [199, 200]. Arginine methylation is abundant in these cells as well as in peripheral naïve T cells [16], all of which have comparable levels of PRMT5 expression; conversely, PRMT5 levels are lower in regulatory T cells [199]. PRMT5 expression is upregulated nearly 4-fold upon stimulation of CD4+and CD8+cells. In mice, PRMT5 deficiency did not affect thymic development of CD4+or CD8+cells but substantially reduced CD4+/CD8+naïve, effector, and central memory T-cell counts in peripheral tissues (spleen, liver, lymph nodes, and bone marrow). PRMT5-depletion did, however, result in cell-intrinsic defects in early iNK T-cell differentiation. Mechanistically, constitutive splicing of both *IL2RG* (γc) and *Jak3* pre-mRNA was impaired by PRMT5 depletion [199]. *ILRG2* exon 6 encodes the γc transmembrane domain, suggesting a PRMT5-dependent regulatory mechanism that potentially adjusts the receptor-to-soluble γc-ratio to establish or maintain T-cell identities. This study also found that PRMT5 expression was downregulated by the Forkhead box transcription factor 3 (FOXP3), the activity of which was shown to be mediated by arginine methylation, as summarized next.

Thymus-derived T-regulatory (tT_{reg}) cell establishment and maintenance is reliant on FOXP3 expression, which is repressed in peripherally-derived pT_{reg}-cells (reviewed [201]). FOXP3 positively regulates the constitutive expression of the high-affinity IL-2 receptor chain α (IL-2Rα, also known as CD25) and negatively regulates IL-2 expression to establish the T_{reg}-cell transcriptional signature [201]. In FOXP3+T-cells, inhibition of PRMT1 downregulates CD25 and upregulates of IL-2 expression, suggesting that arginine methylation influences FOXP3 function [202]. Genes associated with T-helper (T_h)-cell signatures were upregulated in PRMT1-inhibited FOX3P+cells. Reciprocal IPs identified a FOXP3/PRMT1 interaction, and inhibition of PRMT1 decreased both FOXP3 Rme1 and Rme2a. R48 and R51 were identified as PRMT1 substrates by mutational analysis. In FOXP3- cells, expression of wild type FOXP3 produced the CD25/IL-2 expression signature; double R48/51A FOXP3 mutation was required to inhibit the CD25/IL-2 phenotype. In irradiated mice, PRMT1 inhibition or expression of R48/51A in FOX3P-transduced cells resulted in fatal weight loss; mice with wild-type FOX3P cells were asymptomatic in this regard. These results show that PRMT1-dependent arginine methylation of FOXP3 mediates its gene-regulatory activity, which promotes T_{reg}-cell maintenance by inhibiting expression of genes associated with T_h-cell signatures. Additionally, PRMT5 has been implicated in B-cell differentiation and maintenance [203]. These studies highlight an important role for arginine methylation in adaptive immune system development.

Actin and neuronal development

In the brain, the branched webbing of neuronal networks is underpinned by the structured entanglement of the filamentous actin cytoskeleton. Protein Cordon Blue (COBL) is an actin nucleator highly enriched in the brain and is critical for neuronal morphogenesis. COBL is methylated and regulated by PRMT2 [204]. The PRMT2 SH3 domain interacts with a disordered region of the COBL C-terminus (Fig. 2). IHC showed enrichment of PRMT2 in dendritic tress of rat hippocampal neurons (rHCNs) that overlapped with COBL at dendritic growth cones. coIP with PRMT2-SH3-domain peptide precipitated endogenous COBL. In rHCNs, PRMT inhibition significantly reduced dendritic counts

and branching. Conversely, overexpression of PRMT2 or COBL increased both parameters, whereas inhibition of methylation in rHCNs overexpressing PRMT2 neutralized these increases. Similar decreases were observed in PRMT2-knockdown rHCNs, in which ectopic expression of wild-type PRMT2, but not catalytic mutants, rescued dendritic counts and branching. Rme was detected on GFP-COBL immunoprecipitated from rHCNs. COBL contains three intrinsically disordered C-terminal WASP homology (WH2) domains which fold upon binding to G-actin [204, 205]. Arginine methylation occurs near these actin-nucleating WH2 domains at the COBL C-terminus. The observation that PRMT2-dependent arginine methylation within COBL WH2 IDR domains enhances actin-binding highlights the neuronal development importance of arginine methylation mediating both intrinsic disorder and protein-protein interactions.

Metabolic flux

A distinguishing characteristic of cancer cells is their metabolic switch from oxidative phosphorylation to high-flux anaerobic glycolysis (termed the Warburg effect) [206]. Accordingly, glycolytic enzymes are commonly upregulated in cancers [207], including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in metastatic hepatic tissues [208]. Arginine methylation of GAPDH R234 was identified in recent characterizations of the methylome [11, 12]. CARM1 methylation of GAPDH has inhibitory effects on glycolysis; furthermore, GAPDH methylation was suppressed in a panel of human liver cancers [209]. Rme1 and Rme2a were detected on FLAG-GAPDH purified from HEK cells. Enzymatic assays showed that GAPDH from AdOx-treated cells was ~ 50% more active than untreated, suggesting that R234me inhibits GAPDH activity. Consistent with this idea, GAPDH isolated from Adox-treated cells showed a 30% reduction for nicotinamide adenine dinucleotide (NAD +) K_m (Michaelis constant), suggesting that R234me reduces GAPDH substrate affinity. Likewise, R234F (methyl mimic) mutant exhibited a 10-fold increase in glyceraldehyde-3-phosphate (G3P) K_m, and reduced GAPDH activity by a striking 80%. CARM1 methylates GAPDH R234 in vitro, which also inhibited GAPDH activity. In liver cancer cell lines, glucose starvation upregulated CARM1 protein, increased GAPDH R234me, and decreased GAPDH activity. Conversely, when glucose was added to culture medium, CARM1 levels decreased, R234me diminished, and GAPDH activity increased by ~ 50%. These experiments reveal a mechanistic link between GAPDH arginine methylation and glucose levels in liver cancer cells, which may play a critical role in maintaining high-flux glycolysis, driving the Warburg effect.

SIRT7, glucose sensing, and mitochondrial biogenesis

SIRT7 is a histone H3K18-specific deacetylase [210]. Proteomic analyses discovered SIRT7 R388me in a region important for its deacetylase activity [12, 16]. R388 is conserved from flies to humans, suggesting that modification has functional significance. To characterize R388me, SIRT7 was expressed in and purified from HEK cells, and immunoblots detected both Rme1 and Rme2a on SIRT7. AdOx treatment and SIRT7 R388F/K mutation markedly reduced both methylation signals, suggesting that R388 is the predominant site of methylation [211]. Using a SIRT7 R388me-specific antibody that detects both Rme1 and Rme2a, immunoblots revealed that ~ 50% of endogenous SIRT7 is methylated in HEK and L02 (human fetal liver) cells, which was significantly reduced by AdOx treatment. In vitro methylation assays and coIPs identified PRMT6 as the primary SIRT7 methyltransferase. Both wild-type and SIRT7 R388 K effectively deacetylated H3 K18ac when expressed in HEK and MEF cells; however, R388F failed to do so, suggesting that Rme inhibits SIRT7 deacetylase activity. Consistent with this idea, deacetylation of HEK cell-extracted chromatin with in vitro methylated SIRT7 showed significantly increased H3K18ac compared to unmodified SIRT7. Consistently, H3K18ac levels were severely attenuated by SIRT7 purified from H3K cells either co-expressing catalytically inactive PRMT6 or depleted for PRMT6, demonstrating that SIRT7 deacetylase activity is suppressed by PRMT6-dependent Rme. Deacetylation of H3K18 promotes transcriptional silencing of genes implicated in mitochondrial maintenance [212]. SIRT7 knockdown significantly increased mitochondria, oxygen consumption, and ATP production in MEFs [211]. Addback of wild-type SIRT7, but not methyl-mimetic R388F, fully restored mitochondrial mass and metabolic flux. Acetylation at several SIRT7-target gene promoters markedly increased upon PRMT6 inhibition, whereas mitochondrial mass, ATP levels, and oxygen consumption all decreased. These effects were not observed in PRMT6-inhibited SIRT7-knockdown cells. Together, these results indicate that PRMT6-dependent SIRT7 R388me promotes mitochondrial biogenesis and maintenance.

Depletion of glucose in culture media resulted in hypomethylation of SIRT7 coincident with a sharp rise in AMPK phosphorylation, suggesting that activated AMPK inhibits SIRT7 methylation under low-glucose conditions. Consistent with this hypothesis, AICAR-induced activation of AMPK reduced SIRT7 R388me, whereas AMPK knockout, even under glucose starvation, increased SIRT7 methylation. In addition, PRMT6 inhibition in AMPK knockout cells abrogated SIRT7 methylation, and PRMT6-SIRT7 association was mitigated upon glucose starvation or AICAR treatment by coIP. These experiments strongly indicate that AMPK

signals glucose availability to SIRT7 through PRMT6. In fasting mice, upon intraperitoneal glucose administration, SIRT7 methylation increased while AMPK phosphorylation decreased in liver tissues. These results define an AMPK-PRMT6-SIRT7 axis, connecting glucose sensing to arginine methylation and mitochondrial biogenesis. Mechanistically, elevated glucose stimulates AMPK activation that signals inhibition of SIRT7 HDAC activity via PRMT6-dependent SIRT7 R388 methylation. In this manner, H3K18ac is maintained, resulting in expression of genes that drive mitochondrial biogenesis and homeostasis.

Viral replication and reactivation

PRMTs can be co-opted or inhibited by viruses during viral replication [213] and reactivation [214]. West Nile virus (WNV) replication depends on cyclization of its RNA genome (WNV RNA) [213]. hnRNP D (also known as AU-rich element binding protein 1, AUF1, p45 isoform) facilitates RNA cyclization. PRMT1 is necessary for GAR motif methylation within the disordered AUF1 C-terminus (Fig. 2, hnRNP D). Depletion of PRMT1 inhibited AUF1 methylation and decreased WNV replication. Conversely, both cyclization of WNV RNA and replication were most efficient with methylated AUF1. Circular dichroism demonstrated that AUF1 has a high degree of disorder; Rme2a conformationally structures AUF1. Furthermore, AUF1 Rme2a displayed significantly increased affinity for WNV RNA 5′- and 3′-UTRs, and AUF1 Rme2a enhanced WNV RNA replication. Gel-shift assays determined AUF1 Rme2a facilitated more efficient RNA interactions at 5′- and 3′-ends. Fluorescence-based RNA-restructuring assays demonstrated that Rme2a significantly enhances AUF1 RNA 3′-stem-loop melting—a prerequisite for RNA cyclization. A novel function for AUF1 in annealing WNV RNA cyclization sequences was discovered; however, a FRET-based assay suggested both unmodified and AUF Rme2a hybridize cyclization sequences with comparable efficiency. Nonetheless, this report describes how WNV commandeers PRMT1 to promote efficient replication of its genome.

Reactivation of the Kaposi's sarcoma-associated herpesvirus (KSHV) genome involves chromatin remodeling assisted by PRMT5 [214]. The host chromatin environment surrounding latent KSHV viral genome is predominantly repressed. Upon viral reactivation, histone modifying enzymes, including PRMT5, are appropriated to restructure chromatin into a state amenable for transcription of viral RNA. Expression of viral RNA was significantly higher in PRMT5-depleted reactivated KSHV cells, and ChIP-qPCR showed a decrease in H4R3me2s, indicating that PRMT5 suppresses viral transcription through H4R3me2s. During viral reactivation, KSHV protein ORF59 binds to and inhibits the catalytic domain of PRMT5, resulting in loss of H4R3me2s and accumulation of H3K4me3, thereby 'opening' chromatin for viral replication. Thus, viral inhibition of PRMTs promotes transformation of KSHV from latent to lytic infection.

Circulating Rme2a: a biomarker of endothelial dysfunction

L-Arginine is the precursor of nitric oxide (NO). NO is an important vasodilator: endothelial cells of the inner vessels use the enzyme nitric oxide synthase (NOS) to convert a molecule of L-Arg into NO. NO then diffuses from endothelial cells to the smooth muscle cells surrounding blood vessels, resulting in vasodilation, prompting decreased blood pressure and inflammation [215]. As a result of normal proteolysis, methylarginines are released into biological fluids. Circulating Rme2a competes with L-Arg for the NOS catalytic site, inhibiting NO synthesis [216]. Circulating Rme2a levels have emerged as biomarkers for endothelial dysfunction and are associated with cardiovascular disease risk in patients with rheumatoid arthritis (RA). Targeting Rme2a metabolism is, therefore, a potential RA therapy. Thus far, owing to variability in results in inter-study comparisons, reduction of Rme2a and beneficial effects on cardiovascular outcomes are inconclusive [217].

Perspective

Liquid–liquid phase separation is a phenomenon of widespread and recent profound interest. It has largely been investigated with respect to RNA granule formation and function. A large proportion of the proteins implicated in RNA granule phase separation are known targets for arginine methylation (e.g., FUS, hnRNP A2, Lsm4). Similar themes are beginning to emerge in how different levels of interphase genome architecture are compacted and functionally organized into topologically associated domains, compartments, and chromosomal territories. One compelling hypothesis is that transcriptionally active and inactive regions are organized dependent on the degree of phase separation of the chromatin fiber. Consistent with this hypothesis is the observation that transcription factors and RNAP2 cluster into hubs at active gene loci and enhancer regions and promote phase separation [218–221]. Intriguingly, numerous chromatin-associated proteins are arginine methylated, such as histones, transcription factors (e.g., FOXP3 [202], STAT1 [222], E2F-1 [223]), and RNAP2. In light of observations showing RNA granule phase behavior is regulated by arginine methylation, this suggests a similar mechanistic role for arginine methylation in mediating phase transitions critical for the dynamic three-dimensional organization and function of the eukaryotic genome.

We have explored only a sample of the broad-reaching cellular consequences of arginine methylation in Eukarya. Over the past 50 years, methylarginine has staked its claim as a modification of great significance on the PTM frontier. Manifesting in crosstalk with other PTMs, functionalizing IDRs, and mediating LLPS, methylarginine plays a key role in regulating biophysical processes throughout the cell. As we look toward the future, our understanding of cellular physiology will continue to grow as mechanisms governing phase separation and compartmentalization of biological processes are uncovered. Arginine methylation and demethylation have made an early appearance in this arena of disorder-to-order transition and will likely continue to guide our knowledge forward.

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