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JmjC Histone Demethylases: Beyond Histone Lysine Demethylation

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Abstract: Jumonji C histone lysine demethylases (JmjC-KDMs) are key chromatin regulators best known for catalysing histone lysine demethylation. There is growing evidence that JmjC-KDMs have a broader catalytic scope. This review summarises recent advances on JmjC-KDM activities beyond histone lysine demethylation, including arginine demethylation and arginine hydroxylation. We discuss how emerging insights into sequence-reactivity and inter-domain relationships, combinatorial post-translational modifications (PTMs), and cellular context shape substrate selectivity and enzymatic outcomes. These findings highlight substantial mechanistic flexibility within the JmjC-KDM family and may help prompt reconsideration of how their biochemistry is connected to physiological roles. We discuss implications for JmjC-KDM inhibitor development and outline outstanding questions, guiding future research concerning their roles in epigenetic regulation.

Keywords: Arginine demethylation / hydroxylation · Cancer metabolism · Epigenetics · JmjC histone demethylases · JmjC-KDMs · 2-oxoglutarate (2OG) dependent oxygenases

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Research in ***Chris Schofield*** (CJS)'s laboratory centres on contributing to an organic chemistry-based understanding of systems of biological importance, including mechanisms of antibiotic resistance and the regulation of genetic machinery. An important aspect of his work has concerned enabling the exploitation of basic science results for societal/medicinal benefit, including for treatment of infectious diseases and hypoxia related diseases (*e.g.* anaemia and cancer). Two current major research fields of the group are antibiotics/antibiotic resistance and the biochemistry and biology of oxygenases and hypoxia.

Akane Kawamura's group research focuses on understanding the chemistry of epigenetic regulation, developing chemical probes against epigenetic modifying enzymes, and the development of cyclic peptide-based target validation approaches. After obtaining her MChem and DPhil degrees at the University of Oxford, she worked in the biotech sector contributing to early drug discovery projects across a wide range of therapeutic areas. She later returned to academia and established her research group in the Department of Chemistry at the University of Oxford. Akane joined Newcastle University in 2019 as Professor of Chemical Biology and remains a visiting professor at Oxford.

1. Introduction

Molecular studies on epigenetic regulation have focused on how nucleic acid modifications and post-translational modifications (PTMs) regulate gene expression without altering the underlying DNA sequence. Although such modifications, in particular DNA methylation and histone modifications, are now well-known to influence transcription and heritable phenotypes, the underlying biochemistry enabling their introduction and maintenance is far from being understood in detail; further research is needed to clarify their roles in normal healthy development and in complex diseases.[1] PTMs to histones, in particular their *N*-terminal tails, including lysine and arginine methylation, lysine acetylation and ubiquitination, and phosphorylation, are central to epigenetic regulation of chromatin in eukaryotes. Numerous histone-modifying enzymes and proteins that add, read, and / or remove these marks so influencing chromatin structure and gene accessibility have been identified.[2]

Dysregulation of the finely tuned, but normally robust, epigenetic balance contributes to a range of diseases, including developmental disorders and cancer, where abnormal PTM patterns frequently coexist with underlying genetic alterations. In part because many epigenetic-related nucleic acid and protein modifications are potentially reversible, they offer promising opportunities for therapeutic intervention. Although progress has been made in our understanding of the biochemistry of epigenetics and drugs targeting epigenetic regulation have been approved for cancer treatment, it is probably accurate to state that the therapeutic potential of epigenetics remains largely untapped, including from the potential of interpreting and exploiting genomic data.[3] Histone modifying enzymes are validated therapeutic targets as demonstrated by clinically used inhibitors of histone deacetylases and histone methyltransferases.[4] Other histone modifying enzymes,

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including histone demethylases KDM1/LSD1 and KDM4s, are the subject of on-going medicinal chemistry effort.[5]

In this brief review, we focus on the Jumonji-C (JmjCs) histone lysine demethylases (KDMs), a family of oxygenases that have established roles in the removal of N^{ε}-methyl groups on lysine residues on histone tails.[8] Since their discovery approximately 20 years ago, JmjC-KDMs have been found to accept a broad range of the substrates and to catalyse reactions other than N^{ε}-methyl lysine demethylations. These discoveries expand the enzymatic roles of JmjC-KDMs beyond the regulation of histone lysine methylation states, with potential consequences for their roles in diverse cellular processes linked to healthy biology and disease.

2. Histone Lysine Methylation and JmjC-KDMs

Lysine residues on histone tails serve as regulatory sites and undergo diverse PTMs (Fig. 1). While lysine acetylation is generally linked to euchromatin and transcriptional activation, lysine N^{ε}-methylation can either repress or activate transcription, depending on the site and the degree of methylation (mono-, di-, or tri-methylation). For instance, methylation of histone H3 lysine residues K9 and K27 (H3K9, H3K27), and histone H4 residue K20 (H4K20) typically mark heterochromatin and transcriptional silencing, whereas methylation at histone H3 residues K4, K36 and K79 (H3K4, H3K36, H3K79) correlates with transcriptional activation and elongation.[9] Along with that of the guanidino group of histone arginine residues, *N*-methylation of lysine residues plays a key role in recruiting and regulating epigenetic protein complexes and transcription factors.[9b,10] Lysine N^{ε}-methylation dynamics are controlled by methyltransferases (MTs) and KDMs that, respectively, add and remove these marks; changes in lysine N^{ε}-methylation can alter chromatin structure in ways that reshape transcriptional programmes, ultimately influencing cell identity and fate.[9b,10b,11]

There are two major families of KDMs, classified according to their structures and enzymatic mechanisms: the flavin-dependent amine oxidase KDM1 family and Fe(II)/2-oxoglutarate (2OG)-dependent JmjC-KDMs. The first histone-demethylase for which a protein sequence was identified, KDM1A/LSD1, validated the pioneering work showing that histone methylation is a reversible modification.[12] Following the identification of factor inhibiting hypoxia inducible factor (FIH), a JmjC-fold 2OG oxygenase as an asparagine C-3 hydroxylase, playing a role in the hypoxia inducible factor (HIF) mediated response to hypoxia,[13] the JmjC-KDM subfamily was identified.[14] JmjC-KDMs may have evolved from the more extensive family of Fe(II)/2OG oxygenases, which are present in organisms ranging from humans to bacteria.[12a,12b,15] The JmjC demethylase and JmjC hydroxylase nomenclature is used because the JmjC hydroxylases give stable alcohol products,[15c] but this is a little misleading because demethylation likely proceeds *via* hydroxylation and, as we shall see, some JmjC-KDMs give stable alcohol products (Fig. 2). The JmjC-KDMs and hydroxylases also have distinctive structural features.[15c] Hence, although the JmjC demethylase / hydroxylase nomenclature is imperfect, we suggest it is best to continue to use it – at least whilst the functions of JmjC-enzymes are still emerging.

Like other 2OG-dependent oxygenases, members of the JmjC-KDM subfamily have a conserved distorted double-stranded β-helix (DSBH) core fold, comprised of eight β-strands and surrounding elements. The precise nature of the latter defines the 2OG oxygenase structural subfamily, *e.g.* JmjC-KDM or hydroxylase subfamily.[15c] The DSBH core fold supports binding of the single Fe(II) cofactor, which is coordinated by a highly, but not universally conserved HXD/E...H triad of residues. At least in general terms, JmjC-KDM catalysis is proposed to proceed *via* a consensus 2OG oxygenase mechanism.[16] Thus, active site binding of the 2OG co-substrate, which complexes the Fe(II), is followed by that of the substrate, then, finally, O_2. Oxidative decarboxylation of 2OG then leads to the formation of CO_2, Fe-coordinating succinate, and a reactive Fe(IV)=O species, which hydroxylates the substrate. In the case of demethylation, the hydroxylated N^{ε}-methyl group forms an unstable hemiaminal intermediate. The latter fragments give the demethylated product and formaldehyde (Fig. 2).[8,15c,17]

JmjC-KDMs can be classified into subfamilies based on sequence homology and domain architecture. Approximately 20 JmjC-KDM enzymes are reported to be present in humans (Fig. 3).[17a] In addition to the catalytic JmjC-domain, JmjC-KDMs often contain additional ancillary domains, such as reader modules (*e.g.* Tudor domains, PHD-finger domains) and DNA-binding regions that mediate chromatin targeting, protein-protein interactions and recruitment of regulatory complexes (Fig. 3).[12c] Both catalytic and non-catalytic mechanisms contribute to the diverse regulatory functions of the JmjC-KDMs. The JmjC-KDMs have been implicated in numerous diseases, including various cancers, where they are frequently overexpressed or mutated, as well as in developmental and neurological disorders (see Ref. 5 for review).

JmjC-KDM subfamilies (and subfamily members) often display distinct, though sometimes overlapping, substrate and product selectivities (Fig. 3).[5] For example, while KDM2/3/7 subfamily members catalyse demethylation of di- (Kme2) and mono-methyllysines (Kme1), KDM4/5/6 subfamily members can additionally catalyse the demethylation of tri-methyllysines (Kme3). Whilst demethylation at the H3K4 site is primarily catalysed by the KDM5 subfamily, several members of the KDM3/4/7 subfamilies can act on the H3K9 site. The 'established' histone demethylase functions associated with each JmjC-KDM subfamily members are summarised in Fig. 3.[8,19] Despite the abundance of methylations found at different lysine sites across histones (Fig. 1), to date, JmjC-KDM activity has been validated at relatively few lysines on histone tails in cells and *in vivo*, in particular H3K4, H3K9, H3K27, H3K36 and H4K20 (Fig. 3). It is possible other histone demethylases and other histone substrates will be identified; for example, H3K79 methylation has a key role in regulating transcriptional elongation, DNA damage responses and cell cycle control.[20] Although DOT1L methyltransferase, responsible for H3K79 methylation, has been well characterised,[21] a demethylase enzyme capable of acting on this mark has not yet been confirmed.

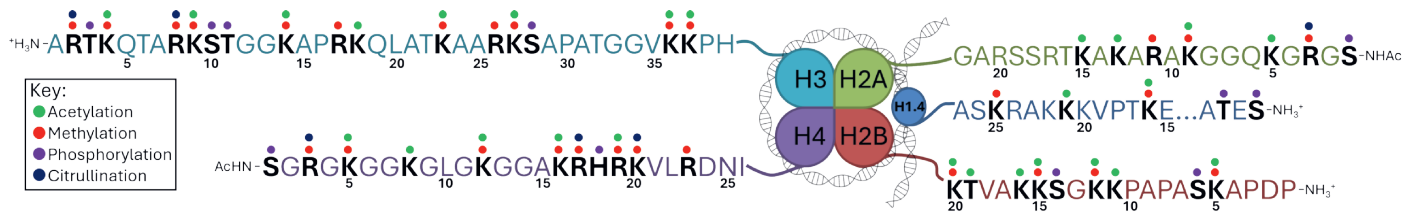


Fig. 1. Diagrammatic representation of some key post-translational modifications found on the histone (H1.4, H2a, H2b, H3, and H4) N-terminal tails.[7]

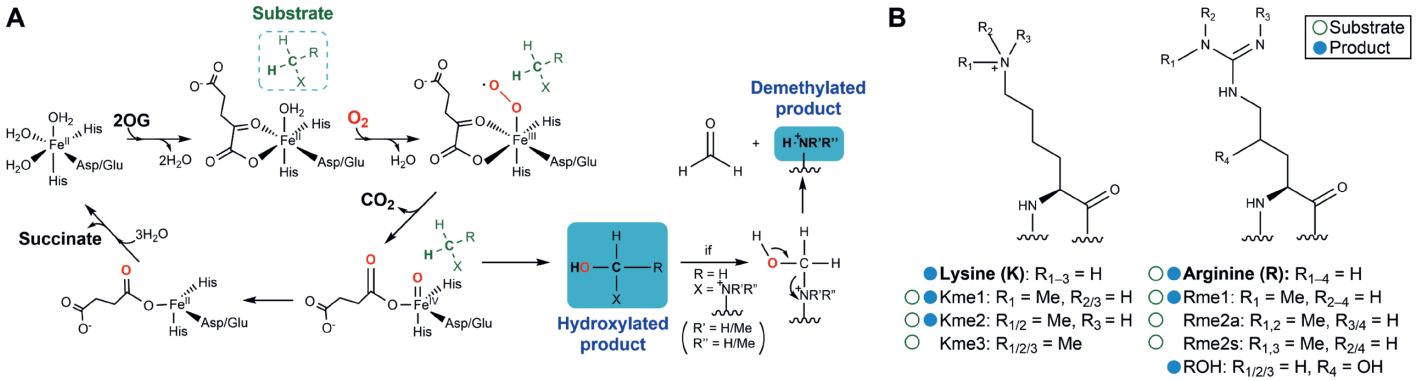


Fig. 2. JmjC-KDM catalysed reactions. (A) Proposed outline of the JmjC-KDMs catalytic mechanism. 2OG binds to the active site Fe(II) in a bidentate manner with consequent loss of two water molecules. Substrate binding is followed by that of O_2, resulting in oxidative decarboxylation of 2OG to give succinate, CO_2, and an Fe(IV)=O intermediate. Rebound-hydroxylation of the N^{ε}-methyl group gives an unstable hemiaminal product that fragments to produce formaldehyde and the demethylated product. Note variations of the consensus 2OG oxygenase mechanism can occur, both in terms of the nature of intermediates and rates of individual steps. Structural regions beyond the immediate active site are also important in catalysis.[18] (B) Substrates and products of JmjC-KDM catalysis.

In vitro studies on isolated catalytic domains of JmjC-KDMs proteins have provided insight into the substrate selectivities of JmjC-KDMs at the level of individual proteins. In some cases, mass-spectrometry based histone peptide screening for JmjC-activities, combined with orthogonal biochemical assays and biophysical investigations, have revealed a flexible substrate scope for JmjC-KDMs, extending beyond their established substrates. Isolated KDM4s were found to demethylate H3K27me3/2 peptides, in addition to their 'established' methylated H3K9 and H3K36 substrates.[19r,22] The KDM4 subfamily also catalyses the demethylation of H1.4K25 (sometimes noted as K26), with kinetics comparable to those observed for H3K9.[19i,19r] In both cases, enzyme-substrate complex crystal structures revealed a similar binding geometry of the residues being demethylated to that observed for H3K9me3 binding.[23] Note, however, that there are differences in the binding modes of the H3K9 and H3K36 substrates to KDM4A,[23] and studies reveal the importance of sequence context in determining reaction outcomes – see below. Further, the crystallographic studies likely underestimate the importance of dynamic motions, both in catalysis and selectivity, a proposal supported by computational studies.[18]

More recent *in vitro* studies have identified additional demethylation activities of the KDM4s on isoforms of the linker histone H1 - H1.2K25, H1.2K26, H1.3K24, H1.4K25, and H1.5K26.[19i,24] KDM4D is also reported to catalyse the demethylation of chemically synthesised histone H3 containing H3K79me3.[25] Although these observations require further cellular validation, they demonstrate the catalytic potential of JmjC-KDMs to demethylate a broader number of N^{ε}-methylated lysine-containing histone peptide sequences than was once perceived.

Interestingly, substrate-analogue studies have shown that some JmjC-KDMs can catalyse the oxidative removal of *N*-alkyl groups other than methyl, such as N^{ε}-ethyl and isopropyl groups on lysines, and demethylate lysine analogue N^{δ}-dimethyl ornithine,[17d] indicating flexibility in the substrate and the C-H binding geometry within the active site. These observations are of interest given the expanding range of histone modifications that are being identified.[26]

Emerging evidence also shows the importance of combinatorial PTMs and non-catalytic ancillary domains of JmjC-KDMs in determining substrate selectivity. For example, the presence of H3K4me3 enhances the KDM4A/C activity at H3K9me3, whereas H3T11 phosphorylation inhibits it, and H3K14ac has mixed effects on demethylation activity at H3K9me3.[27] In KDM5 and KDM7 subfamilies, plant homeobox domain (PHD)-finger domains recognise the H3K4 methylation status to direct / allosterically modulate the KDM activities of JmjC-domains.[28] KDM7B PHD-finger binds to H3K4me3 and directs the productive binding of the KDM7B JmjC domain to H3K9me2 to substantially enhance its demethylation rate; by contrast, with KDM7A, PHD binding to H3K4me3 hinders H3K9me2 demethylation, so promoting H3K27me2 demethylation.[28d] In KDM5A, a set of PHD-fingers has been shown to coordinate the binding to its substrate H3K4me3 (PHD-finger 3) and its product H3K4me0 (PHD-finger 1) to enhance the JmjC-KDM activity, suggesting operation of a positive-feedback mechanism to propagate demethylation along the chromatin.[28d]

PTMs to JmjC-KDMs themselves, including phosphorylation, are reported to affect their functions, by changing their sub-cellular localisation and chromatin binding, complex formation/dissociation, protein stability or KDM activities.[29] On the basis of cellular studies KDM7C has been reported to have H3K9me2 demethylase activity when phosphorylated at S1056, as catalysed by Protein Kinase A (PKA)[19p] or at S655 by adenosine monophosphate (AMP)-activated protein kinase (AMPK).[30] KDM7C, however, has an atypical Fe(II)-binding mode, with a Tyr at the position corresponding to second iron-binding His, and has been considered 'enzymatically inactive' as an isolated protein.[19m] Enhancement of H3K9me2 demethylation activity was also observed when S884 of KDM7B is phosphorylated by cyclin E and cyclin-dependent kinase 2 complex (Cyclin E-CDK2).[31] Together, these and other observations illustrate how substrate sequences, histone PTM patterns, ancillary domains, and enzyme specific PTMs have roles in regulating JmjC-KDM activity in a context dependent manner.

3. JmjC-KDM Substrates Beyond N^{ε}-Methyl Lysine Residues

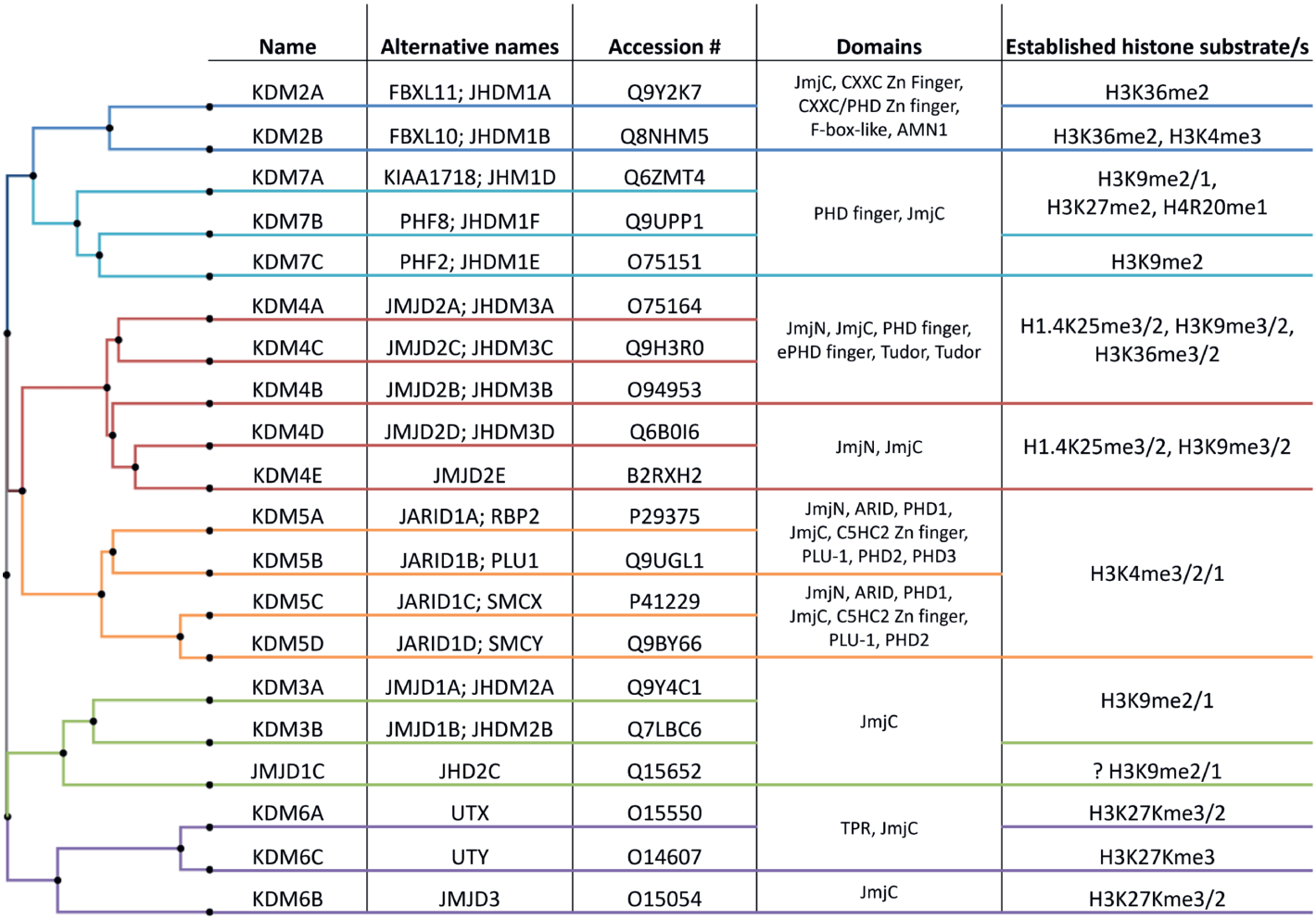
In vitro substrate-analogue studies on JmjC-KDMs, inspired by the broad selectivities of 2OG oxygenases in microbes,[16b] further highlight the broader catalytic potential of JmjC-KDMs. Perhaps most importantly, these studies led to the discovery of arginine demethylase (RDM) activities of JmjC KDMs on H3 and H4 peptides, challenging the long-held view of some that arginine methylation by protein arginine methyltransferases (PRMTs) is irreversible.[32] *N*-Methylation of one, or both, of the terminal nitrogens of the arginine guanidino group is a chemically stable PTM, with di-methylation occurring in both symmetric and asymmetric modes (Fig. 2). Detecting and quantifying arginine methylation in cells is technically challenging, complicating studies investigating the operation of *N*-methylated arginine demethylation.[32–33] Peptidyl-arginine deiminases (PADs), such as human PAD2 and

PAD4, hydrolyse arginine to citrulline (Fig. 1).[34] PAD4 can also act on mono-methyl arginine *in vitro*, though this has not been confirmed in cells. Since citrulline (as far as is known) cannot be converted to arginine and since PAD and PRMT enzymes target overlapping substrates, citrullination is considered irreversible and mutually exclusive with respect to arginine-methylation.[34] Although JMJD6 was initially reported to catalyse arginine demethylation of several histone and non-histone substrates,[35] subsequent studies have shown that JMJD6 is a protein lysyl C-5 hydroxylase.[36]

Using isolated proteins, several JmjC-KDMs, including KDM4E, KDM5C, KDM3A, and KDM6B, have since been shown to catalyse demethylation of a range of mono- and di-methylated (symmetric and asymmetric) arginine residues in both histones and non-histone peptide substrates, with mechanistic studies supporting a 2OG-dependent oxidative demethylation pathway analogous to that of N^{ε}-methyl lysyl demethylation (Fig. 2).[32] No RDM activity has been observed with the KDM2/7 subfamily members tested.[32] Recent work has expanded the list of RDM-competent enzymes to include KDM5A–D and possibly KDM4A–E, though KDM4D shows only weak activity. To date, JmjC-KDM–catalysed arginine demethylation has been observed on histones H2A, H3, and H4, including of H3R2me2a, H3R8me2s/a, H3R17me2a, H3R26me2a, H4R3me2a, H2AR3me2a, and H4R3me2s.[10c,10d,32] Distinct substrate preferences have been observed for the tested JmjC-KDMs with RDM activities indicating that *N*-methyl arginine demethylation, like N^{ε}-methyl lysine demethylation, is selective and context dependent. Several studies have subsequently reported JmjC-KDM dependent changes in cellular arginine methylation on both histone and non-histone proteins.[37]

The available structural and mechanistic information indicates the RDM catalysis proceeds by an analogous mechanism proposed for that for KDM catalysis (Fig. 2).[32] However, the factors involved in determining whether, or not, a JmjC-KDM has RDM activity are poorly understood and it should be noted that in general RDM activities are lower than those observed for KDM catalysis.[10c,10d,32] There is also a possibility that some JmjC oxygenases specialise in, or only have, RDM activity.

A notable recent observation was that KDM4E, and, to a lesser extent, KDM4D, also function as a C4 arginine hydroxylase acting on H2A R20 in peptides and calf histones.[10d,32] Hydroxylation was not observed when the *N*-terminal residues (1–9) of the H2A fragment substrate were removed, a striking contrast to efficient KDM4 KDM activity on relatively short H3 peptides,[10d,19c] indicating that hydroxylation at R20 requires substrate recognition at the H2A N-terminus. The extent of hydroxylation at R20 was



Name	Alternative names	Accession #	Domains	Established histone substrate/s
KDM2A	FBXL11; JHDM1A	Q9Y2K7	JmjC, CXXC Zn Finger, CXXC/PHD Zn finger, F-box-like, AMN1	H3K36me2
KDM2B	FBXL10; JHDM1B	Q8NHM5		H3K36me2, H3K4me3
KDM7A	KIAA1718; JHM1D	Q6ZMT4	PHD finger, JmjC	H3K9me2/1, H3K27me2, H4R20me1
KDM7B	PHF8; JHDM1F	Q9UPP1		
KDM7C	PHF2; JHDM1E	O75151		H3K9me2
KDM4A	JMJD2A; JHDM3A	O75164	JmjN, JmjC, PHD finger, ePHD finger, Tudor, Tudor	H1.4K25me3/2, H3K9me3/2, H3K36me3/2
KDM4C	JMJD2C; JHDM3C	Q9H3R0		
KDM4B	JMJD2B; JHDM3B	O94953		
KDM4D	JMJD2D; JHDM3D	Q6B0I6	JmjN, JmjC	H1.4K25me3/2, H3K9me3/2
KDM4E	JMJD2E	B2RXH2		
KDM5A	JARID1A; RBP2	P29375	JmjN, ARID, PHD1, JmjC, C5HC2 Zn finger, PLU-1, PHD2, PHD3	H3K4me3/2/1
KDM5B	JARID1B; PLU1	Q9UGL1		
KDM5C	JARID1C; SMCX	P41229	JmjN, ARID, PHD1, JmjC, C5HC2 Zn finger, PLU-1, PHD2	
KDM5D	JARID1D; SMCY	Q9BY66		
KDM3A	JMJD1A; JHDM2A	Q9Y4C1	JmjC	H3K9me2/1
KDM3B	JMJD1B; JHDM2B	Q7LBC6		
JMJD1C	JHD2C	Q15652		? H3K9me2/1
KDM6A	UTX	O15550	TPR, JmjC	H3K27Kme3/2
KDM6C	UTY	O14607		H3K27Kme3
KDM6B	JMJD3	O15054	JmjC	H3K27Kme3/2

Fig. 3. Phylogenetic tree of main JmjC-KDM subfamilies based on full-length sequences, together with alternative names, domains, and known histone substrates of each JmjC-KDM.[24] Sequence alignments were carried out using the MAFFT online tool and the phylogenetic tree was built using the Phylo.io online tool. Domain information was collected using the NCBI Batch Web CD-Search Tool. The KDM subfamilies are coloured differently: KDM2s, KDM3s, KDM4s, KDM5s, KDM6s, and KDM7s. JARID = Jumonji/AT-rich interaction domain-containing protein; KDM = lysine demethylase; JHDM = Jumonji-C domain-containing histone demethylase; JmjC = Jumonji C; JMJD = Jumonji domain-containing protein; JmjN = Jumonji-N; UTX/Y = ubiquitously transcribed tetratricopeptide repeat, X/Y chromosome; PHF2/8 = plant homeobox domain finger protein 2/8; RBP2 = retinoblastoma binding protein 2; AMN1 = antagonist of mitotic exit network 1; PHD = plant homeodomain; ARID = AT-rich interaction domain; and TPR = tetratricopeptide repeat. See Refs. [5,14a] for more detailed phylogenetic analyses. Adapted from Ref. [7].

comparable to the RDM activity at R3 and was apparently unaffected by H2A R3 N-methylation status but was substantially reduced when R3 was substituted with alanine, suggesting potential PTM crosstalk involving hydroxylation. These results further highlight the importance of sequence identity in the extent to which KDM4E acts as a KDM, RDM, or arginine hydroxylase. Previously a JmjC hydroxylase, JMJD5, catalysing C3-arginine hydroxylation of a non-histone substrate has been identified.[10d] The KDM4 work represents the first demonstration of C4-arginyl hydroxylase activity by a JmjC-KDM, or other, oxygenase. As an aside these results highlight the potential of engineered JmjC oxygenases for modification of lysine residues in proteins of therapeutic interest, including antibodies.

4. Non-Histone Substrates of the JmjC-KDMs

Demethylation of proteins other than histones has historically been difficult to study, in part due to the small 14 Da mass shift of methylation, minimal charge differences between methylated and unmethylated residues, the low levels of some of the proteins of interest, and the scarcity of highly specific antibodies.[38] Despite these challenges, thousands of non-histone lysine and arginine methylation sites have been reported, many on transcription-related proteins,[39] and non-histone methylation is now recognised as a major regulator of apoptosis, transcription, protein synthesis, and cell-cycle progression.[39a,39f,40] Evidence suggests that non-histone methylation is likely reversible, as it is for histones, but in most cases the responsible KDMs/RDMs have not been identified. A limited number of non-histone substrates have been reported for JmjC-KDMs to date; these include p65 (K218/K221)[41] and β-catenin(K) lysine demethylation for KDM2A,[42] ARID5B(K336) lysine demethylation for KDM7B,[19p] and arginine demethylation of PI3KC2α(R175) by KDM4A in cells.[37b] *In vitro* activities on methylated peptides have been reported using isolated JmjC-KDMs including lysine demethylation by KDM4A–C on EHMT2(K185), CDYL1(K135), WIZ(K305), CSB(K170/K297/K448/K1054), ACINUS(K654), HDAC1(K432), DNMT1(K70) and KLF12(K313);[43] by KDM3C on KDM6B(K991), p300(K1774) and KMT2A(K1534);[44] and by KDM5A on MTA1(K532).[45] RDM activity has been observed on G3BP1(R447/R460) peptides by KDM5C/D.[10c] Many of the reported non-histone demethylase substrate proteins often carry multiple PTMs, reinforcing the idea that modifications operate within interconnected signalling networks.

5. Factors that Influence Enzyme Activity: Oxygen and other Co-factors; KDM Inhibitors and Oncometabolites

Most JmjC-KDM inhibitors identified to date act as 2OG-competitive ligands that coordinate the catalytic Fe(II). These include the broad-spectrum 2OG oxygenase inhibitors *N*-oxalylglycine (NOG),[46] IOX1,[47] and 2,4-pyridine dicarboxylic acid (2,4-PDCA),[48] as well as individual JmjC-KDM and sub-family selective inhibitors, such as GSK-J1/4,[49] CPI-455,[19j] KDM5C-49,[50] KDOAM25,[51] and TACH101 (a KDM4 subfamily selective anticancer drug that has been in clinical development).[51] This 2OG competitive strategy builds on the progress made in early work on 2OG oxygenase inhibitors for HIF stabilisation (now used clinically[52] and in agrochemical applications of 2OG oxygenase inhibitors).[53] However, 2OG competitive inhibitors have the potential to inhibit many JmjC-KDM reactions, including both the KDM and RDM activities as well as other activities, as shown by studies on KDM4E where the arginine hydroxylation activity was also inhibited by 2OG competitors.[54] Thus, the epigenetic changes observed in cells following treatment with these inhibitors may reflect the combined inhibitory effects of multiple JmjC-KDM functions. Substrate-competitive inhibitors offer a means to disentangle the distinct functions of JmjC-KDMs by preferentially targeting specific substrate interactions. In this regard, cyclic peptide inhibitors represent a potential strategy for the highly selective and potent inhibition of specific sets of JmjC-KDMs, although their applications are currently limited by poor cell permeability.[55] It is also worth noting that in a cellular context, endogenous metabolites that compete with 2OG, such as TCA cycle intermediates / related compounds levels of which can be elevated in cancer (*e.g.* 2HG), and (localised) oxygen availability may directly influence JmjC-KDM activities, including in a substrate dependent manner.[55]

6. Conclusions

There is very strong evidence for important roles of the JmjC-KDMs in healthy biology and diseases, with their roles in cancer being of particular interest from a therapeutic perspective. To date, however, no drugs targeting JmjC-KDMs have been approved. At least in part, this may reflect incomplete knowledge of their roles, including in terms of the reactions they catalyse. Following identification of 2OG oxygenases that catalyse protein hydroxylation during collagen biosynthesis in animals, work on them and related enzymes in microbes and plants demonstrated that they can catalyse a very wide range of oxidative reactions, sometimes with the same enzyme catalysing multiple reaction types.[16b] For a substantial period known 2OG oxygenases catalysed reactions in animals were limited to hydroxylations, that is, the formation of stable alcohols. The discovery of the JmjC-KDMs was an interesting one from the oxygenase perspective, because it opened the possibility that they catalyse a wider range of oxidative reactions than had previously been perceived. Recent work on the JmjC-KDMs has revealed their active sites manifest substantial flexibility, supporting catalysis involving KDM, RDM, and hydroxylation reactions. It seems quite likely that other types of reactions will be discovered for them, potentially involving completely new PTMs. The discovery of diverse new JmjC-KDM substrates highlights the need for further investigation into their substrate-binding modalities, substrate selectivities, and catalytic mechanisms, with validation of these activities in *in vivo* contexts being an important challenge. The recent findings highlighted in this review reveal JmjC-KDM functions extend well beyond histone lysine demethylation and current nomenclature may ultimately require reconsideration.

Because JmjC-KDMs play key roles in eukaryotic gene regulation and are therapeutic targets of interest, most JmjC-KDM inhibitors developed to date will likely affect all their activities, in some cases involving KDM, RDM and hydroxylase reactions, raising concerns regarding potential off-target side effects, including with respect to non-histone substrates, underscoring the importance of deeper mechanistic insight.

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Author Contributions

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[1] a) C. T. Wu, J. R. Morris, *Science* **2001**, *293*, 1103, https://doi.org/10.1126/science.293.5532.1103; b) C. D. Allis, T. Jenuwein, in 'Nat Rev Genet', Vol. 17, 2016/06/28 Ed. Laboratory of Chromatin Biology and Epigenetics, The Rockefeller University, 1230 York Avenue, New York 10065, New York, USA. Max Planck Institute of Immunobiology and Epigenetics, Stubeweg 51, Freiburg D-79108, Germany., **2016**, p. 487,

https://doi.org/10.1038/nrg.2016.59; c) S. B. Baylin, P. A. Jones, *Nat. Rev. Cancer* **2011**, *11*, 726, https://doi.org/10.1038/nrc3130.

[2] a) C. Dupont, D. R. Armant, C. A. Brenner, *Semin. Reprod. Med.* **2009**, *27*, 351, https://doi.org/10.1055/s-0029-1237423; b) R. Marmorstein, R. C. Trievel, *Biochim. Biophys. Acta* **2009**, *1789*, 58, https://doi.org/10.1016/j.bbagrm.2008.07.009.

[3] a) K. Struhl, *Cell* **1999**, *98*, 1, https://doi.org/10.1016/S0092-8674(00)80599-1; b) P. A. Jones, S. B. Baylin, *Nat. Rev. Genet.* **2002**, *3*, 415, https://doi.org/10.1038/nrg816; c) P. A. Jones, S. B. Baylin, *Cell* **2007**, *128*, 683, https://doi.org/10.1016/j.cell.2007.01.029; d) P. A. Jones, P. W. Laird, *Nat. Genet.* **1999**, *21*, 163, https://doi.org/10.1038/5947; e) A. P. Feinberg, R. Ohlsson, S. Henikoff, *Nat. Rev. Genet.* **2006**, *7*, 21, https://doi.org/10.1038/nrg1748; f) P. A. Jones, R. Martienssen, *Cancer Res.* **2005**, *65*, 11241, https://doi.org/10.1158/0008-5472.CAN-05-3865; g) C. B. Yoo, P. A. Jones, *Nat. Rev. Drug Discovery* **2006**, *5*, 37, https://doi.org/10.1038/nrd1930.

[4] W. Dai, X. Qiao, Y. Fang, R. Guo, P. Bai, S. Liu, T. Li, Y. Jiang, S. Wei, Z. Na, X. Xiao, D. Li, *Signal Transduction Targeted Ther.* **2024**, *9*, 332, https://doi.org/10.1038/s41392-024-02039-0.

[5] Z. H. Gray, M. A. Honer, P. Ghatalia, Y. Shi, J. R. Whetstine, *Cell*, **2025**, *188*, 1747, https://doi.org/10.1016/j.cell.2025.02.023.

[6] H. Huang, B. R. Sabari, B. A. Garcia, C. David Allis, Y. Zhao, *Cell* **2014**, *159*, 458, https://doi.org/10.1016/j.cell.2014.09.037.

[7] J. Bonnici, University of Oxford, PhD Thesis, **2020**.

[8] Y.-i. Tsukada, J. Fang, H. Erdjument-Bromage, M. E. Warren, C. H. Borchers, P. Tempst, Y. Zhang, *Nature* **2006**, *439*, 811, http://www.nature.com/nature/journal/v439/n7078/suppinfo/nature04433_S1.html;

[9] a) G. E. Zentner, S. Henikoff, *Nat. Struct. Mol. Biol.* **2013**, *20*, 259; b) E. L. Greer, Y. Shi, *Nat. Rev. Genet.* **2012**, *13*, 343, https://doi.org/10.1038/nrg3173; c) A. Portela, M. Esteller, *Nat. Biotechnol.* **2010**, *28*, 1057, https://doi.org/10.1038/nbt.1685.

[10] a) T. Kouzarides, *Cell* **2007**, *128*, 693, https://doi.org/10.1016/j.cell.2007.02.005; b) C. T. Walsh, S. Garneau-Tsodikova, G. J. Gatto Jr., *Angew. Chem. Int. Ed.* **2005**, *44*, 7342, https://doi.org/10.1002/anie.200501023; c) J. Bonnici, R. Oueini, E. Salah, C. Johansson, C. J. Schofield, A. Kawamura, *FEBS Lett.* **2023**, *597*, 933, https://doi.org/10.1002/1873-3468.14586; d) J. Bonnici, R. Oueini, E. Salah, C. Johansson, E. Pires, M. Abboud, R. S. Dawber, A. Tumber, P. Rabe, H. Sarac, C. J. Schofield, A. Kawamura, *Commun. Biol.* **2024**, *7*, 1583, https://doi.org/10.1038/s42003-024-07183-5.

[11] a) C. Sawan, Z. Herceg, *Adv. Genet.* **2010**, *70*, 57, https://doi.org/10.1016/b978-0-12-380866-0.60003-4; b) C. C. Thinnes, K. S. England, A. Kawamura, R. Chowdhury, C. J. Schofield, R. J. Hopkinson, *Biochim. Biophys. Acta* **2014**, *1839*, 1416, https://doi.org/10.1016/j.bbagrm.2014.05.009.

[12] a) Y. Shi, F. Lan, C. Matson, P. Mulligan, J. R. Whetstine, P. A. Cole, R. A. Casero, Y. Shi, *Cell* **2004**, *119*, 941, https://doi.org/10.1016/j.cell.2004.12.012; b) F. Forneris, C. Binda, M. A. Vanoni, A. Mattevi, E. Battaglioli, *FEBS Lett.* **2005**, *579*, 2203, https://doi.org/10.1016/j.febslet.2005.03.015; c) Y. Shi, J. R. Whetstine, *Mol. Cell* **2007**, *25*, 1, https://doi.org/10.1016/j.molcel.2006.12.010.

[13] K. S. Hewitson, L. A. McNeill, M. V. Riordan, Y. M. Tian, A. N. Bullock, R. W. Welford, J. M. Elkins, N. J. Oldham, S. Bhattacharya, J. M. Gleadle, P. J. Ratcliffe, C. W. Pugh, C. J. Schofield, *J. Biol. Chem.* **2002**, *277*, 26351, https://doi.org/10.1074/jbc.C200273200.

[14] a) P. M. Clissold, C. P. Ponting, *Trends Biochem. Sci.* **2001**, *26*, 7, https://doi.org/10.1016/S0968-0004(00)01700-X; b) Y. Tsukada, J. Fang, H. Erdjument-Bromage, M. E. Warren, C. H. Borchers, P. Tempst, Y. Zhang, 'Nature', Vol. 439, 2005/12/20 ed., Howard Hughes Medical Institute, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolin, 27599-7295, USA., **2006**, p. 811, https://doi.org/10.1038/nature04433.

[15] a) Y. J. Shi, C. Matson, F. Lan, S. Iwase, T. Baba, Y. Shi, *Mol. Cell*, **2025**, *19*, 857, https://doi.org/10.1016/j.molcel.2005.08.027; b) R. Chowdhury, R. Sekirnik, N. C. Brissett, T. Krojer, C. H. Ho, S. S. Ng, I. J. Clifton, W. Ge, N. J. Kershaw, G. C. Fox, J. R. C. Muniz, M. Vollmar, C. Phillips, E. S. Pilka, K. L. Kavanagh, F. von Delft, U. Oppermann, M. A. McDonough, A. J. Doherty, C. J. Schofield, *Nature* **2014**, *510*, 422, https://doi.org/10.1038/nature13263; c) S. Markolovic, T. M. Leissing, R. Chowdhury, S. E. Wilkins, X. Lu, C. J. Schofield, *Curr. Opin. Struct. Biol.* **2016**, *41*, 62, https://doi.org/10.1016/j.sbi.2016.05.013.

[16] a) J. E. Baldwin, R. M. Adlington, M. A. Russell, C. J. Schofield, M. E. Wood, *J. Labelled Compd. Radiopharm.* **1989**, *27*, 1091, https://doi.org/10.1002/jlcr.2580270914; b) R. P. Hausinger, in '2-Oxoglutarate-Dependent Oxygenases', Eds. C. Schofield, R. Hausinger, The Royal Society of Chemistry, **2015**, p. 0, https://doi.org/10.1039/9781782621959-00001.

[17] a) R. J. Klose, E. M. Kallin, Y. Zhang, *Nat. Rev. Genet.* **2006**, *7*, 715, http://www.nature.com/nrg/journal/v7/n9/suppinfo/nrg1945_S1.html; b) L. J. Walport, R. J. Hopkinson, C. J. Schofield, *Curr. Opin. Chem. Biol.* **2012**, *16*, 525, https://doi.org/10.1016/j.cbpa.2012.09.015; c) R. J. Hopkinson, R. B. Hamed, N. R. Rose, T. D. Claridge, C. J. Schofield, *ChemBioChem* **2010**, *11*, 506, https://doi.org/10.1002/cbic.200900713; d) R. J. Hopkinson, L. J. Walport, M. Munzel, N. R. Rose, T. J. Smart, A. Kawamura, T. D. Claridge, C. J. Schofield, *Angew. Chem. Int. Ed.* **2013**, *52*, 7709, https://doi.org/10.1002/anie.201303282.

[18] S. Devadas, M. G. Thomas, S. B. J. S. Rifayee, B. Varada, W. White, E. Sommer, K. Campbell, C. J. Schofield, C. Z. Christov, *Chem. -Eur. J.* **2025**, *31*, e202403989, https://doi.org/10.1002/chem.202403989.

[19] a) K. Yamane, C. Toumazou, Y. i. Tsukada, H. Erdjument-Bromage, P. Tempst, J. Wong, Y. Zhang, *Cell* **2006**, *125*, 483, https://doi.org/10.1016/j.cell.2006.03.027; b) J. R. Whetstine, A. Nottke, F. Lan, M. Huarte, S. Smolikov, Z. Chen, E. Spooner, E. Li, G. Zhang, M. Colaiacovo, Y. Shi, *Cell* **2006**, *125*, 467, https://doi.org/10.1016/j.cell.2006.03.028; c) L. Hillringhaus, W. W. Yue, N. R. Rose, S. S. Ng, C. Gileadi, C. Loenarz, S. H. Bello, J. E. Bray, C. J. Schofield, U. Oppermann, *J. Biol. Chem.* **2011**, *286*, 41616, https://doi.org/10.1074/jbc.M111.283689; d) J. Christensen, K. Agger, P. A. C. Cloos, D. Pasini, S. Rose, L. Sennels, J. Rappsilber, K. H. Hansen, A. E. Salcini, K. Helin, *Cell* **2007**, *128*, 1063, https://doi.org/10.1016/j.cell.2007.02.003; e) S. Iwase, F. Lan, P. Bayliss, L. de la Torre-Ubieta, M. Huarte, H. H. Qi, Johnathan, R. R. Whetstine, A. Bonni, T. M. Roberts, Y. Shi, *Cell* **2007**, *128*, 1077, https://doi.org/10.1016/j.cell.2007.02.017; f) S. H. Hong, Y. W. Cho, L. R. Yu, H. Yu, T. D. Veenstra, K. Ge, *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *285*, 9322, https://doi.org/10.1074/jbc.C109.097667; g) P. Trojer, J. Zhang, M. Yonezawa, A. Schmidt, H. Zheng, T. Jenuwein, D. Reinberg, *J. Biol. Chem.* **2009**, *284*, 8395, https://doi.org/10.1074/jbc.M807818200; h) R. J. Klose, Q. Yan, Z. Tothova, K. Yamane, H. Erdjument-Bromage, P. Tempst, D. G. Gilliland, Y. Zhang, W. G. Kaelin, *Cell* **2007**, *128*, 889, https://doi.org/10.1016/j.cell.2007.02.013; i) J. R. Horton, A. Engstrom, E. L. Zoeller, X. Liu, J. R. Shanks, X. Zhang, M. A. Johns, P. M. Vertino, H. Fu, X. Cheng, *J. Biol. Chem.* **2016**, *291*, 2631, https://doi.org/10.1074/jbc.M115.698449; j) K. Yamane, K. Tateishi, R. J. Klose, J. Fang, L. A. Fabrizio, H. Erdjument-Bromage, J. Taylor-Papadimitriou, P. Tempst, Y. Zhang, *Mol. Cell* **2007**, *25*, 801, https://doi.org/10.1016/j.molcel.2007.03.001; k) K. Agger, P. A. C. Cloos, J. Christensen, D. Pasini, S. Rose, J. Rappsilber, I. Issaeva, E. Canaani, A. E. Salcini, K. Helin, *Nature* **2007**, *449*, 731, https://doi.org/10.1038/nature06145; l) L. J. Walport, R. J. Hopkinson, M. Vollmar, S. K. Madden, C. Gileadi, U. Oppermann, C. J. Schofield, C. Johansson, *J. Biol. Chem.* **2014**, *289*, 18302, https://doi.org/10.1074/jbc.M114.555052.

[20] M. Ljungman, L. Parks, R. Hulbatte, K. Bedi, *Mutat. Res., Rev. Mutat. Res.* **2019**, *780*, 48, https://doi.org/10.1016/j.mrrev.2017.11.001.

[21] O. Arnold, K. Barbosa, A. J. Deshpande, N. Zhu, *Front. Cell Dev. Biol.* **2022**, *10*, 917125, https://doi.org/10.3389/fcell.2022.917125.

[22] S. T. Williams, L. J. Walport, R. J. Hopkinson, S. K. Madden, R. Chowdhury, C. J. Schofield, A. Kawamura, *Epigenetics* **2014**, *9*, 1596, https://doi.org/10.4161/15592294.2014.983381.

[23] S. S. Ng, K. L. Kavanagh, M. A. McDonough, D. Butler, E. S. Pilka, B. M. R. Lienard, J. E. Bray, P. Savitsky, O. Gileadi, F. Von Delft, N. R. Rose, J. Offer, J. C. Scheinost, T. Borowski, M. Sundstrom, C. J. Schofield, U. Oppermann, *Nature* **2007**, *448*, 87, https://doi.org/10.1038/nature05971.

[24] V. A. Türkmen, A. Tumber, E. Salah, S. Kaur, C. J. Schofield, J. Mecinović, *RSC Chem. Biol.* **2025**, *6*, 1607, https://doi.org/10.1039/d5cb00083a.

[25] M. Jbara, N. Guttmann-Raviv, S. K. Maity, N. Ayoub, A. Brik, *Bioorg. Med. Chem.* **2017**, *25*, 4966, https://doi.org/10.1016/j.bmc.2017.04.015.

[26] W. Yao, X. Hu, X. Wang, *Signal Transduction Targeted Ther.* **2024**, *9*, 232, https://doi.org/10.1038/s41392-024-01918-w.

[27] B. Lohse, C. Helgstrand, J. B. L. Kristensen, U. Leurs, P. A. C. Cloos, J. L. Kristensen, R. P. Clausen, *PLoS ONE* **2013**, *8*, 67653, https://doi.org/10.1371/journal.pone.0067653.

[28] a) J. Cao, Z. Liu, W. K. Cheung, M. Zhao, S. Y. Chen, S. W. Chan, C. J. Booth, D. X. Nguyen, Q. Yan, *Cell Rep.* **2014**, *6*, 868, https://doi.org/10.1016/j.celrep.2014.02.004; b) J. R. Horton, A. K. Upadhyay, H. H. Qi, X. Zhang, Y. Shi, X. Cheng, *Nat. Struct. Mol. Biol.* **2010**, *17*, 38, https://doi.org/10.1038/nsmb.1753; c) B. J. Klein, L. Piao, Y. Xi, H. Rincon-Arano, S. B. Rothbart, D. Peng, H. Wen, C. Larson, X. Zhang, X. Zheng, M. A. Cortazar, P. V. Pena, A. Mangan, D. L. Bentley, B. D. Strahl, M. Groudine, W. Li, X. Shi, T. G. Kutateladze, *Cell Rep.* **2014**, *6*, 325, https://doi.org/10.1016/j.celrep.2013.12.021; d) I. O. Torres, K. M. Kuchenbecker, C. I. Nnadi, R. J. Fletterick, M. J. Kelly, D. G. Fujimori, *Nat. Commun.* **2015**, *6*, 6204, https://doi.org/10.1038/ncomms7204.

[29] N. M. Karakatsanis, J. J. Hamey, M. R. Wilkins, *Trends Biochem. Sci.* **2024**, *49*, 257, https://doi.org/10.1016/j.tibs.2023.12.004.

[30] Y. Dong, H. Hu, X. Zhang, Y. Zhang, X. Sun, H. Wang, W. Kan, M.-j. Tan, H. Shi, Y. Zang, J. Li, *Signal Transduction Targeted Ther.* **2023**, *8*, 95, https://doi.org/10.1038/s41392-022-01302-6.
[31] L. Sun, Y. Huang, Q. Wei, X. Tong, R. Cai, G. Nalepa, X. Ye, *J. Biol. Chem.* **2015**, *290*, 4075, https://doi.org/10.1074/jbc.M114.602532.
[32] L. J. Walport, R. J. Hopkinson, R. Chowdhury, R. Schiller, W. Ge, A. Kawamura, C. J. Schofield, *Nat. Commun*. **2016**, *7*, 11974, https://doi.org/10.1038/ncomms11974, http://www.nature.com/articles/ncomms11974#supplementary-information.
[33] a) A. Böttger, M. S. Islam, R. Chowdhury, C. J. Schofield, A. Wolf, *Biochem. J.* **2015**, *468*, 191, https://doi.org/10.1042/BJ20150278; b) J. Bonnici, University of Oxford, **2024**.
[34] a) E. R. Vossenaar, A. J. W. Zendman, W. J. Van Venrooij, G. J. M. Pruijn, *BioEssays* **2003**, *25*, 1106, https://doi.org/10.1002/bies.10357; b) K. Nakashima, T. Hagiwara, M. Yamada, *J. Biol. Chem*. **2002**, *277*, 49562, https://doi.org/10.1074/jbc.M208795200; c) T. Hagiwara, K. Nakashima, H. Hirano, T. Senshu, M. Yamada, *Biochem. Biophys. Res. Commun.* **2002**, *290*, 979, https://doi.org/10.1006/bbrc.2001.6303.
[35] a) Y. Wang, J. Wysocka, J. Sayegh, Y. H. Lee, J. R. Pertin, L. Leonelli, L. S. Sonbuchner, C. H. McDonald, R. G. Cook, Y. Dou, R. G. Roeder, S. Clarke, M. R. Stallcup, C. D. Allis, S. A. Coonrod, *Science* **2004**, *306*, 279, https://doi.org/10.1126/science.1101400; b) P. R. Thompson, W. Fast, *ACS Chem. Biol*. **2006**, *1*, 433, https://doi.org/10.1021/cb6002306; c) G. L. Cuthbert, S. Daujat, A. W. Snowden, H. Erdjument-Bromage, T. Hagiwara, M. Yamada, R. Schneider, P. D. Gregory, P. Tempst, A. J. Bannister, T. Kouzarides, *Cell* **2004**, *118*, 545, https://doi.org/10.1016/j.cell.2004.08.020; d) H. Denis, R. Deplus, P. Putmans, M. Yamada, R. Métivier, F. Fuks, *Mol. Cell. Biol.* **2009**, *29*, 4982, https://doi.org/10.1128/mcb.00285-09.
[36] a) B. Chang, Y. Chen, Y. Zhao, R. K. Bruick, *Science* **2007**, *318*, 444, https://doi.org/10.1126/science.1145801; b) C. Poulard, J. Rambaud, N. Hussein, L. Corbo, M. Le Romancer, *PLoS ONE* **2014**, *9*, e87982, https://doi.org/10.1371/journal.pone.0087982; c) P. Lawrence, J. S. Conderino, E. Rieder, *Virology* **2014**, *452-453*, 1, https://doi.org/10.1016/j.virol.2013.12.040; d) W. W. Gao, R. Q. Xiao, B. L. Peng, H. T. Xu, H. F. Shen, M. F. Huang, T. T. Shi, J. Yi, W. J. Zhang, X. N. Wu, X. Gao, X. Z. Lin, P. C. Dorrestein, M. G. Rosenfeld, W. Liu, *Proc. Natl. Acad. Sci. U. S. A*. **2015**, *112*, E3327, https://doi.org/10.1073/pnas.1509658112.
[37] a) S. Li, S. Ali, X. Duan, S. Liu, J. Du, C. Liu, H. Dai, M. Zhou, L. Zhou, L. Yang, P. Chu, L. Li, R. Bhatia, D. E. Schones, X. Wu, H. Xu, Y. Hua, Z. Guo, Y. Yang, L. Zheng, B. Shen, *Cell Rep*. **2018**, *23*, 389, https://doi.org/10.1016/j.celrep.2018.03.051; b) Y. Cho, J. W. Hwang, M. T. Bedford, D. H. Na, D.-G. Song, S.-N. Kim, Y. K. Kim, *Cell Commun. Signaling* **2025**, *23*, 409, https://doi.org/10.1186/s12964-025-02419-1.
[38] a) D. Levy, *Cell. Mol. Life Sci*. **2019**, *76*, 2873, https://doi.org/10.1007/s00018-019-03142-0; b) K. K. Biggar, S. S. Li, *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 5, https://doi.org/10.1038/nrm3915.
[39] a) D. Levy, A. J. Kuo, Y. Chang, U. Schaefer, C. Kitson, P. Cheung, A. Espejo, B. M. Zee, C. L. Liu, S. Tangsombatvisit, R. I. Tennen, A. Y. Kuo, S. Tanjing, R. Cheung, K. F. Chua, P. J. Utz, X. Shi, R. K. Prinjha, K. Lee, B. A. Garcia, M. T. Bedford, A. Tarakhovsky, X. Cheng, O. Gozani, *Nat. Immun*. **2011**, *12*, 29, https://doi.org/10.1038/ni.1968; b) S. Ko, J. Ahn, C. S. Song, S. Kim, K. Knapczyk-Stwora, B. Chatterjee, *Mol. Endocrinol*. **2011**, *25*, 433, https://doi.org/10.1210/me.2010-0482; c) H. Liu, M. Galka, E. Mori, X. Liu, Y. f. Lin, R. Wei, P. Pittock, C. Voss, G. Dhami, X. Li, M. Miyaji, G. Lajoie, B. Chen, S. S. C. Li, *Mol. Cell* **2013**, *50*, 723, https://doi.org/10.1016/j.molcel.2013.04.025; d) S. M. Carlson, K. E. Moore, E. M. Green, G. M. Martín, O. Gozani, *Nat. Protoc.* **2014**, *9*, 37, https://doi.org/10.1038/nprot.2013.164; e) T. Y. Lee, C. W. Chang, C. T. Lu, T. H. Cheng, T. H. Chang, *Comput. Biol. Chem*. **2014**, *50*, 11, https://doi.org/10.1016/j.compbiolchem.2014.01.009; f) Q. Xie, Y. Bai, J. Wu, Y. Sun, Y. Wang, Y. Zhang, P. Mei, Z. Yuan, *J. Recepet. Signal Trans*duction **2011**, *31*, 139, https://doi.org/10.3109/10799893.2011.552914; g) M. V. Botuyan, J. Lee, I. M. Ward, J. E. Kim, J. R. Thompson, J. Chen, G. Mer, *Cell* **2006**, *127*, 1361, https://doi.org/10.1016/j.cell.2006.10.043; h) P. K. Mazur, N. Reynoird, P. Khatri, P. W. T. C. Jansen, A. W. Wilkinson, S. Liu, O. Barbash, G. S. Van Aller, M. Huddleston, D. Dhanak, P. J. Tummino, R. G. Kruger, B. A. Garcia, A. J. Butte, M. Vermeulen, J. Sage, O. Gozani, *Nature* **2014**, *510*, 283, https://doi.org/10.1038/nature13320.
[40] a) A. Dhayalan, S. Kudithipudi, P. Rathert, A. Jeltsch, *Chem. Biol*. **2011**, *18*, 111, https://doi.org/10.1016/j.chembiol.2010.11.014; b) G. Martin, A. Ostareck-Lederer, A. Chari, N. Neuenkirchen, S. Dettwiler, D. Blank, U. Rüegsegger, U. Fischer, W. Keller, *RNA* **2010**, *16*, 1646, https://doi.org/10.1261/rna.2164210; c) M. Jansson, S. T. Durant, E. C. Cho, S. Sheahan, M. Edelmann, B. Kessler, N. B. La Thangue, *Nat. Cell Biol*. **2008**, *10*, 1431, https://doi.org/10.1038/ncb1802; d) J. Huang, J. Dorsey, S. Chuikov, X. Zhang, T. Jenuwein, D. Reinberg, S. L. Berger, *J. Biol. Chem.* **2010**, *285*, 9636, https://doi.org/10.1074/jbc.M109.062588.
[41] T. Lu, M. W. Jackson, B. Wang, M. Yang, M. R. Chance, M. Miyagi, A. V. Gudkov, G. R. Stark, *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 46, https://doi.org/10.1073/pnas.0912493107.
[42] L. Lu, Y. Gao, Z. Zhang, Q. Cao, X. Zhang, J. Zou, Y. Cao, *Dev. Cell* **2015**, *33*, 660, https://doi.org/10.1016/j.devcel.2015.04.006.
[43] a) V. K. C. Ponnaluri, D. T. Vavilala, M. Mukherji, *Biochem. Biophys. Res. Commun*. **2011**, *405*, 588, https://doi.org/10.1016/J.BBRC.2011.01.073; b) V. K. C. Ponnaluri, D. T. Vavilala, S. Putty, W. G. Gutheil, M. Mukherji, *Biochem. Biophys. Res. Commun*. **2009**, *390*, 280, https://doi.org/10.1016/j.bbrc.2009.09.107.
[44] A. Chopra, W. G. Willmore, K. K. Biggar, *Biomolecules* **2022**, *12*, 641, https://doi.org/10.3390/biom12050641.
[45] M. Hoekstra, K. K. Biggar, *Anal. Biochem*. **2021**, *633*, 114429, https://doi.org/10.1016/j.ab.2021.114429.
[46] C. J. Cunliffe, T. J. Franklin, N. J. Hales, G. B. Hill, *J. Med. Chem*. **1992**, *35*, 2652, https://doi.org/10.1021/jm00092a016.
[47] R. J. Hopkinson, A. Tumber, C. Yapp, R. Chowdhury, W. Aik, K. H. Che, X. S. Li, J. B. L. Kristensen, O. N. F. King, M. C. Chan, K. K. Yeoh, H. Choi, L. J. Walport, C. C. Thinnes, J. T. Bush, C. Lejeune, A. M. Rydzik, N. R. Rose, E. A. Bagg, M. A. McDonough, T. J. Krojer, W. W. Yue, S. S. Ng, L. Olsen, P. E. Brennan, U. Oppermann, S. Muller, R. J. Klose, P. J. Ratcliffe, C. J. Schofield, A. Kawamura, *Chem. Sci*. **2013**, *4*, 3110, https://doi.org/10.1039/C3SC51122G.
[48] N. R. Rose, S. S. Ng, J. Mecinović, B. M. R. Liénard, S. H. Bello, Z. Sun, M. A. McDonough, U. Oppermann, C. J. Schofield, *J. Med. Chem*. **2008**, *51*, 7053, https://doi.org/10.1021/jm800936s.
[49] B. Heinemann, J. M. Nielsen, H. R. Hudlebusch, M. J. Lees, D. V. Larsen, T. Boesen, M. Labelle, L.-O. Gerlach, P. Birk, K. Helin, *Nature* **2014**, *514*, E1, https://doi.org/10.1038/nature13688.
[50] C. Johansson, S. Velupillai, A. Tumber, A. Szykowska, E. S. Hookway, R. P. Nowak, C. Strain-Damerell, C. Gileadi, M. Philpott, N. Burgess-Brown, N. Wu, J. Kopec, A. Nuzzi, H. Steuber, U. Egner, V. Badock, S. Munro, N. B. LaThangue, S. Westaway, J. Brown, N. Athanasou, R. Prinjha, P. E. Brennan, U. Oppermann, *Nat. Chem. Biol*. **2016**, *12*, 539, https://doi.org/10.1038/nchembio.2087, http://www.nature.com/nchembio/journal/v12/n7/abs/nchembio.2087.html#supplementary-information.
[51] C. Chandhasin, V. Dang, F. Perabo, J. Del Rosario, Y. K. Chen, E. Filvaroff, J. A. Stafford, M. Clarke, *Anticancer Drugs* **2023**, *34*, 1122, https://doi.org/10.1097/CAD.0000000000001514.
[52] I. C. Macdougall, *Curr. Opin. Nephrol. Hypertens*. **2022**, *31*, 399, https://doi.org/10.1097/mnh.0000000000000813.
[53] a) L. Yan, V. J. Colandrea, J. J. Hale, *Expert Opin. Ther. Pat.* **2010**, *20*, 1219, https://doi.org/10.1517/13543776.2010.510836; b) W. Rademacher, *Annu. Rev. Plant Biol*. **2000**, *51*, 501, https://doi.org/10.1146/annurev.arplant.51.1.501.
[54] a) J. Bonnici, R. Oueini, E. Salah, C. Johansson, E. Pires, M. Abboud, R. S. Dawber, A. Tumber, P. Rabe, H. Sarac, C. J. Schofield, A. Kawamura, *Commun. Biol.* **2024**, *7*, 1583, https://doi.org/10.1038/s42003-024-07183-5. b) J. Bonnici, A. Tumber, A. Kawamura, C. J. Schofield, *Philos. Trans. R. Soc., B* **2018**, *373*, 20170071, https://doi.org/10.1098/rstb.2017.0071.
[55] a) A. Kawamura, M. Munzel, T. Kojima, C. Yapp, B. Bhushan, Y. Goto, A. Tumber, T. Katoh, O. N. King, T. Passioura, L. J. Walport, S. B. Hatch, S. Madden, S. Muller, P. E. Brennan, R. Chowdhury, R. J. Hopkinson, H. Suga, C. J. Schofield, *Nat. Commun*. **2017**, *8*, 14773, https://doi.org/10.1038/ncomms14773; b) M. Y. Zhang, H. Yang, G. Ortiz, M. J. Trnka, N. Petronikolou, A. L. Burlingame, W. F. DeGrado, D. G. Fujimori, *Chem. Sci.* **2022**, *13*, 6599, https://doi.org/10.1039/d2sc00555g; c) O. D. Coleman, J. Macdonald, B. Thomson, J. A. Ward, C. J. Stubbs, T. E. McAllister, S. Clark, S. Amin, Y. Cao, M. I. Abboud, Y. Zhang, H. Sanganee, K. V. M. Huber, T. D. W. Claridge, A. Kawamura, *Chem. Sci.* **2023**, *14*, 7136, https://doi.org/10.1039/D2SC05944D.