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4. Supplementary Datasets

Supplementary dataset 1: Sequences of all animal *Prdm* proteins.

Supplementary dataset 2: Sequences of the non-metazoan proteins.

Supplementary dataset 3: Sequences of the human zinc finger proteins used for the phylogenetic analysis.

Supplementary dataset 4: Sequences of the SET domains used for the phylogenetic analysis.

Supplementary Text

Supplementary Text 1: Structural and functional evolution of the PR domain.

SET domains are thought to mediate the enzymatic activity of a large number of Histone Methyltransferases (HMTs). An intrinsic HMT activity has been described for vertebrate Prdm2, Prdm3, Prdm6, Prdm7/9, Prdm8, Prdm13 and Prdm16 proteins and linked to their derived SET domain, the so called PR domain (Hohenauer and Moore 2012; Pinheiro et al. 2012; Hanotel et al. 2013). To better understand the evolution of the PR domain, we made a multiple alignment of the particular SET domain of a selection of Prdm proteins from several animal species and other SET domains from other human proteins (Fig. S1). We found the three previously reported blocks (blocks *a*, *b* and *c*) of conserved sequences (Hohenauer and Moore 2012). For the three blocks, we were able to slightly extend the size of the conserved block as compared to the previous report (blocks *a'*, *b'* and *c'* in Fig. S1). We also found a fourth conserved block in the N-terminal part of the SET domain (block *d* in Fig. S1). All these blocks show sequence conservation among Prdm proteins (Fig. S2).

We next looked for the potential conservation of residues that have been shown to be important for the HMT activity of SET domain proteins (Figs. S1 and S2) (Dillon et al. 2005; Hohenauer and Moore 2012). The function of the SET domain involves the transfer of a methyl group from S-Adenosyl-L-Methionine (AdoMet) to a lysine residue of the histone protein (Dillon et al. 2005). The AdoMet-binding pocket contains a G-X-G (glycine – any amino acid – glycine) motif located in block *a* and also a N-H (asparagine – histidine) motif located in the conserved R/H-X-X-N-H-X-C box in block *b*. The G-X-G motif is found in some Prdm proteins such as Prdm7/9 which has been shown to bind AdoMet in a similar fashion than other SET domain proteins (Wu et al. 2013). However, in most other proteins only the second G of the G-X-G motif is conserved (including Prdm2 which has been shown to display HMT activity) (Figs. S1 and S2). The N-H motif is very poorly conserved in Prdm proteins: whereas the N residue is found in some Prdm proteins, the H residue is never observed. The cysteine residue of the R/H-X-X-N-H-X-C box, described as being crucial for HMT activity (Rea et al. 2000; Derunes et al. 2005), is only found in two Prdm proteins (Prdm2 and Prdm3/16 from Human). Two important residues for the catalytic activity of SET proteins are two tyrosines (Y): one located in block *a* and the other one in block *c* (Figs. S1 and S2). Whereas the first one is only poorly conserved in Prdm proteins, the other one is found in almost all Prdm proteins. Finally in block *c*, there is a conserved Y (tyrosine) or F (phenylalanine) residue that has been shown to be important to determine whether the SET protein will be a mono-, di- or tri-methylase (Dillon et al. 2005). Whereas different types of methylase activity are found among Prdm proteins (me1, me2 and me3), neither Y nor F residues are found at this particular position in most Prdm proteins (Figs. S1 and S2).

We also looked for additional conserved residues in the PR domain which would likely be important for the function of the proteins (Figs. S1 and S2). We found four residues that are strongly conserved in Prdm and non-Prdm proteins: a G (glycine) in block *a*, I/V (isoleucine or valine) in block *c* and a E-L (glutamic acid – leucine) motif in block *c*. We also found three other residues strongly conserved in Prdm proteins and found in few or very few non-Prdm proteins: a L-P (leucine – proline) motif in block *d* and W (tryptophan) in the R/H-X-X-N-H-X-C box (block *b*). Finally, we found five motifs well conserved in Prdm proteins, but not in non-Prdm ones, F-G-P (phenylalanine – glycine – proline) in block *a*, A (alanine) and E-Q-N-L (glutamic acid – glutamine – asparagine – leucine) in block *b*, Y/F-Y/F (tyrosine / phenylalanine - tyrosine / phenylalanine) in block *c*, and L-V-W (leucine – valine – tryptophan) in block *c*.

Our analysis supports the idea that the PR domain is a highly divergent subtype of SET domains, as many residues strongly conserved among SET domain proteins are poorly or not conserved in Prdm proteins. Therefore, based on the amino acid sequence of the PR domain, it is very difficult to predict whether a given Prdm protein may have an intrinsic HMT activity or not. We also identified several residues that are well conserved in Prdm proteins and could therefore be important for their functions.

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Wu H, Mathioudakis N, Diagouraga B, Dong A, Dombrovski L, Baudat F, Cusack S, de Massy B, Kadlec J. 2013. Molecular basis for the regulation of the H3K4 methyltransferase activity of PRDM9. Cell Rep 5:13–20.

Supplementary Text 2: Phylogenetic analysis of the Prdm subfamilies.

Prdm1 subfamily: *Prdm1* genes are only found in bilaterians (Table S1). Several gene duplications occurred in this subfamily, most of which seem to have occurred in lineages leading to single studied species (Table S1, Fig. S5). For example, three genes are found in the ascidian *Ciona intestinalis* while only one in *Ciona savignyi*, two genes in two nematode species (*Pristionchus pacificus* and *Loa loa*) while only one in the other studied nematodes, two genes in the annelids *Platynereis dumerilii* and *Helobdella robusta* while a single gene in the other annelid *Capitella teleta*, and two genes in *Tetranychus urticae* while only one in all other arthropods. In all but some cases, the two paralogs found in a given species are closer to each other than to their orthologs, further supporting the occurrence of recent lineage specific duplications (Fig. S5). As described in the main text, two duplications likely took place in vertebrates (Fig. 5).

Prdm2 subfamily: Prdm2 genes are only found in few lophotrochozoans and in deuterostomes (Table S1). Few gene duplications occurred during the evolution of the Prdm2 subfamily: one of them occurred during early teleost evolution, which was followed by the loss of one of the two paralogs in *Gasterosteus aculeatus* and *Xiphophorus maculatus* (Figs. 5 and S6).

Prdm3/16 subfamily: *Prdm3/16* genes are only found in bilaterians (Table S1) and underwent several gene duplications in different lineages (Fig. 6). Some duplications occurred in lineages leading to single representative species, such as *Ciona intestinalis*, *Pinctada fucata* and *Helobdella robusta* (Fig. 6). As described in the main text, two duplications probably occurred in arthropods (Fig. 4) and one in vertebrates (Fig. 5).

Prdm4 and Prdm5 subfamilies: *Prdm4* genes are only found in bilaterians and *Prdm5* genes only in vertebrates (Table S1). We did not detect any gene duplications in the Prdm4 and Prdm5 subfamilies (Figs. S7 and S8).

Prdm6 subfamily: *Prdm6* genes are only found in deuterostomes and non-bilaterians (Table S1). Whereas in deuterostomes, sponges, and in the ctenophore *Mnemiopsis leidyi* a single gene per species is found, several genes per species are observed in cnidarians and in the other ctenophore *Pleurobrachia bachei* (Table S1). In the phylogenetic analysis, a few cases of paralogs groupings are observed (Fig. S9). We did not find evidence for an ancient duplication in cnidarians. Therefore, independent duplications in the different cnidarian lineages seem to be the most likely hypothesis.

Prdm7/9 subfamily: *Prdm7/9* genes are found in all the studied metazoan lineages, with the exception of ctenophores and placozoans (Table S1). Several gene duplications occurred, most of which in lineages giving rise to single representative species, such as the cnidarians *Acropora digitifera* and *Hydra magnipapillata*, the annelid *Platynereis dumerilii*, and the vertebrate *Petromyzon marinus* (Fig. S10). A particular case is the echinoderm *Strongylocentrotus purpuratus* in which 10 genes belonging to this subfamily is found, most of which group together (Fig. S10). This indicates an important expansion of this subfamily in this species. Though *Prdm7/9* has been entirely lost in several deuterostome species, a single *Prdm7/9* gene is found in non-vertebrate chordates and in many vertebrates including non-primate mammalians (Table S1). As described in the main text, a gene duplication occurred in the primate lineage, leading to the presence of *Prdm7* and *Prdm9* genes (Fig. 5).

Prdm8 subfamily: Only few gene duplications occurred in the Prdm8 subfamily whose members are found in ctenophores, lophotrochozoans, ecdysozoans, and deuterostomes (Table S1, Fig. S11). As described in the main text, a duplication probably took place in the Gnathostomata lineage (Fig. 5).

Prdm10/15 subfamily: *Prdm10/15* genes are only found in bilaterians (Table S1; Fig. 7). As described in the main text, a gene duplication occurred during early vertebrate evolution (Fig. 5).

Prdm11 subfamily: *Prdm11* genes are found only in vertebrates (Table S1) and we did not detect any gene duplication in this subfamily (Fig. S12).

Prdm12 subfamily: *Prdm12* genes are found in all the studied metazoan lineages, with the exception of ctenophores (Table S1, Fig. S13). A single gene is found in most studied species, except in some teleosts in which two paralogs are found. As described in the main text, a duplication likely occurred in Euteleostei (Fig. 5).

Prdm13 subfamily: *Prdm13* genes are found in cnidarians, placozoans, and bilaterians (Table S1). Only two of the studied species possess two *Prdm13* genes: the cnidarian *Nematostella* *vectensis* and the myriapod *Strigamia maritima* (Table S1, Fig. S14). This suggests independent duplication events.

Prdm14 subfamily: *Prdm14* genes are found in cnidarians, lophotrochozoans, and deuterostomes (Table S1). Several gene duplications occurred, most of which probably in lineages giving rise to single representative species, such as *Nematostella vectensis* (several duplications), *Capitella teleta*, *Saccoglossus kowalevskii*, and *Callorhinchus milii* (Fig. S15). One gene duplication also likely occurred in the lineage leading to the last common ancestor of the mollusks *Crassostrea gigas* and *Lottia gigantea*, followed by additional duplications in the *Crassostrea* lineage (Fig. S15).

Prdm17 subfamily: No gene duplication occurred in this vertebrate-specific subfamily (Table S1, Fig. S16), but this gene was lost in many species, including metatherians and birds (Table S1, Fig. 5).

Supplementary datasets

Supplementary dataset 1: Sequences of all animal *Prdm* proteins.

Amphimedon queenslandica

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>Aque_prdm7_9
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Acropora digitifera

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Caenorhabditis elegans

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Supplementary dataset 2: Sequences of the non metazoan proteins.

Allomyces macrogynus

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Chondrus crispus

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Cryptococcus neoformans

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Dictyostelium discoideum

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Ectocarpus siliculosus

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Emiliania huxleyi

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Fusarium oxysporum

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Gaeumannomyces graminis

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Giardia lamblia

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Gibberella moniliformis

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Guillardia theta

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Monosiga brevicollis

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>Mbre_32717
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Magnaporthe oryzae

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>Mory_12536
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>Mory_03030
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>Mory_15393
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>Mory_12009
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Mortierella verticillata

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DKKKRRDEKE
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Salpingoeca rosetta

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>Sros_8808
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Spizellomyces punctatus

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>Spun_5829
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Tetrahymena thermophila

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>Tthe_EAR96094
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Thalassiosira pseudonana

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>Tpse_Thaps6535
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Toxoplasma gondii

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>Tgon_TGME49_046520
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Trypanosoma brucei

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>Tbru_EAN76378
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>Tbru_EAN79525
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VATEMSPREEYLTWSDGDSPAANSQQTDRVSPMTKTQENRAVSSTGVKSLPVNSDLKPQD
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YIERCERKMREAMRFLDSTNNGSFNVMR
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Supplementary dataset 3: Sequences of the human zinc finger proteins used for the phylogenetic analysis.

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>Hsap_z782
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>Hsap_z16
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>Hsap_z226
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>Hsap_z808
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>Hsap_zSCAN5B
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>Hsap_z112
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Supplementary dataset 4: Sequences of the SET domains used for the phylogenetic analysis.

Allomyces macrogynus

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Saccharomyces cerevisiae

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Sphaeroforma arctica

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