MAD2L2 Promotes Open Chromatin in Embryonic Stem Cells and Derepresses the Dppa3 Locus

Ali Rahjouei,1,2 Mehdi Pirouz,1,4 Michela Di Virgilio,2 Dirk Kamin,3 and Michael Kessel1,*

1RG Developmental Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg, 37077 Göttingen, Germany
2DNA Repair and Maintenance of Genome Stability, Max-Delbruck Center for Molecular Medicine, 13125 Berlin-Buch, Germany
3Department NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, Am Fassberg, 37077 Göttingen, Germany
4Present address: Stem Cell Program, Boston Children’s Hospital and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: mkessel1@gwdg.de

http://dx.doi.org/10.1016/j.stemcr.2017.02.011

SUMMARY

The chromatin of naive embryonic stem cells (ESCs) has a largely open configuration, as evident by the lack of condensed heterochromatin and the hypomethylation of DNA. Several molecular mechanisms promoting this constellation were previously identified. Here we present evidence for an important epigenetic function of MAD2L2, a protein originally known for its role in DNA damage repair, and for its requirement in germ cell development. We demonstrate using super-resolution microscopy that numerous MAD2L2 microfoci are exclusively associated with euchromatin, similar to other factors of the DNA damage response. In the absence of MAD2L2 the amount of heterochromatin demarcated by H3K9me2 was significantly increased. Among the most strongly suppressed genes was Dppa3, an ESC- and germ-cell-specific gene regulating DNA methylation. In Mad2l2-deficient ESCs 5-methylcytosine levels were globally increased, while several imprinted genes became hypomethylated and transcriptionally activated. Our results emphasize the important function of MAD2L2 for the open chromatin configuration of ESCs.

INTRODUCTION

Open chromatin is a global, constitutive feature of naive embryonic stem cells (ESCs), which are characterized by low levels of repressive histones and DNA methylation (Gaspar-Maia et al., 2011; Meshorer and Misteli, 2006). Diverse factors are required to maintain ESC chromatin in a decondensed state. They are important to stabilize pluripotency and to maintain the potential for self-renewal and differentiation. DPPA3 (also called PGC7 or Stella) is one of the factors known to modulate epigenetic processes in both embryonic and germ cell development (Payer et al., 2003). It is a highly specific, maternal effect gene required for early embryonic cells and primordial germ cells (PGCs), and demarcates naive versus primed pluripotency (Hayashi et al., 2008; Ohinata et al., 2008). Heterogeneous expression of DPPA3 was detected in ESC populations growing in Lif/serum as well as Lif/2i medium (Hayashi et al., 2008; Singer et al., 2014). DPPA3 heterogeneity was concluded to result from a combination of low burst frequencies and large burst sizes (Singer et al., 2014). DPPA3 is known to be involved in the maintenance of the low level of DNA methylation typical for early embryonic cells as well as ESCs (Hayashi et al., 2008). At the same time, it protects imprinted loci against demethylation ( Nakamura et al., 2007). DPPA3 is required for the generation of fully reprogrammed pluripotent stem cells, in particular for the erasure of the epigenetic memory encoded in the DNA methylation pattern ( Xu et al., 2015b).

Both ESCs and PGCs, the two cell types characterized by the expression of DPPA3, require the MAD2L2 protein for the proper establishment of their epigenetic status (Pirouz et al., 2013, 2015). In the absence of Mad2L2 PGCs do not reprogram toward a more flexible chromatin configuration after their induction, and ESCs are not able to propagate their typical open chromatin, which is necessary for stable pluripotency.

MAD2L2 (also called REV7 or MAD2B) was originally identified by its function in DNA repair, playing an accessory role in translesion DNA repair and an inhibitory role in the resection of 5' ends after DNA double-strand breaks and at unprotected telomeres (Boersma et al., 2015; Xu et al., 2015a). As a downstream component of the DNA damage response pathway, MAD2L2 promotes non-homologous end joining versus homologous recombination. The role of MAD2L2 in DNA repair, ESC development, and PGC development is tightly connected to the epigenetic status of the respective cells. To investigate the relationship between MAD2L2 and the formation of open chromatin we compared wild-type and Mad2l2-deficient ESCs, employing super-resolution microscopy, transcriptome sequencing, methylome sequencing, and chromatin immunoprecipitation sequencing (ChiP-seq) analysis. We identified Dppa3 as a downstream target of MAD2L2, and derived a model explaining the epigenetic effects of MAD2L2.
RESULTS

Heterogeneous Expression of MAD2L2 in ESCs

ESCs are known to express variable levels of specific RNAs and/or proteins, depending among other factors on growth conditions. This indicates a highly dynamic regulation of gene expression and reflects a metastable equilibrium between distinct cellular states. We characterized the MAD2L2 protein in wild-type ESCs and compared the dynamics with classical ESC markers. Individual cells of a colony expressed significantly different levels of MAD2L2 (Figure 1A and Movie S1), either very high (4.8% out of 1,000 evaluated, cells from five different fields), high (72%), or low (23.2%), resembling the heterogeneity observed for DPPA3 expression (Figure 3A) (Hayashi et al., 2008). In contrast, both MAD2L2-positive and -negative ESCs expressed Oct4 (Figure S1A). The differentiation of ESCs into epiblast-like cells (EpiLC) represents the transition from naïve to primed pluripotency (Hayashi et al., 2011). This was accompanied by a drastic downregulation of the MAD2L2 protein (Figure S1B) and Dppa3 mRNA (Figure 3C). Together, these observations suggest a dynamic fluctuation from a state of naïve pluripotency demarcated by Mad2l2 and Dppa3 expression to a primed state with both markers.

Figure 1. MADL2 Localization in Relation to Open and Closed Chromatin of ESCs

(A) Confocal microscopy of a colony with MAD2L2-positive and -negative ESCs.
(B–F) STED microscopy of ESC sections (B, 50 nm; C–F, 80 nm) were analyzed by immunocytochemistry for (B) DNA, (C) H3K9me2, and (D–F) H3K27me3.
(G) MAD2L2 expression in ESCs overlapped with euchromatin as identified by H3K4me2 (G) and AcH4 (H).
(I) ESC with condensed chromosomes in the metaphase of the cell cycle. The cell was stained for DNA (DAPI, blue), and MAD2L2 (red), and analyzed by confocal microscopy. See also Figures S1 and S2.
(J) Fractionation of ESCs revealed that virtually the complete amount of MAD2L2 protein was in the chromatin fraction (Chr), and not in the cytoplasm (Cyto) or the nuclear fraction remaining after chromatin separation (Nuc).
(K) The numbers of ESCs expressing high levels of H3K9me2 or H3K27me3 were significantly increased in the absence of MAD2L2.

All observations were confirmed with three experimental replicates. Scale bars represent 10 μm (A), 0.86 μm (B–F), 4 μm (G and H), and 10 μm (I).
off. EpilCs can be further converted to unipotent PGC-like cells (PGCLCs), close in vitro equivalents of PGCs, on embryonic day 9 (Hayashi et al., 2011). PGCLCs represent exactly the developmental stage at which a crucial function of MAD2L2 was previously demonstrated (Pirouz et al., 2013). High expression levels of both MAD2L2 and DPPA3 are typical for germ cells including PGCLCs, and for naïve ESCs (Figures S2A and 3C).

Association of MAD2L2 with Open Chromatin/ Euchromatin

In ESCs the MAD2L2 protein was associated with the chromatin fraction, however; it did not decorate condensed, mitotic chromosomes (Figures 1I and 1J). Super-resolution microscopy revealed that it was distributed in microfoci, not overlapping with the DNA-enriched heterochromatin near the nuclear lamina and around the nucleolus, as identified by H3K9me2 (Figures 1B and 1C) (Willig et al., 2006). High levels of MAD2L2 protein were found in many ESCs of a colony in regions separate from transcriptionally inactive chromatin, as identified by H3K27me3 (Figures 1E and 1F). H3K27me3 accumulated in more central areas of ESC nuclei, compared with the peripheral localization of H3K9me2 (Figures 1C and 1E). ESC colonies always additionally contained cells with either predominantly MAD2L2 or predominantly H3K27me3, but not both, proteins (Figures 1D and 1F). More precisely, 70.6% of wild-type ESCs (out of 1,141 evaluated cells, from four fields) predominantly expressed MAD2L2 in the absence of H3K27me3, 23.6% had a non-overlapping expression of both MAD2L2 and H3K27me3, and 5.7% predominantly expressed H3K27me3 in the absence of MAD2L2. These numbers reflect the highly dynamic chromatin status in ESCs. Line scan histogram profiles through single nuclei revealed that the fourth most significantly down-regulated gene was Dppa3 (Figure 3B). Downregulation of this gene was previously accompanied by the re-induction of the Oct4-GFP transgene and by a loss of the high levels of H3K9me2 typical for EpilCs (Figure S2B). However, Mad2l2-deficient PGCLCs maintained high levels of H3K9me2 and were not able to reprogram toward H3K27me3 (Figure S2C). This resembled the initial induction of Mad2l2-deficient PGCs and their defective epigenetic reprogramming observed in vivo (Pirouz et al., 2013). Taken together, we found a correlation between the presence of MAD2L2 and an open chromatin configuration, as is typical for naïve pluripotency and germ cells after their epigenetic reprogramming. In the absence of MAD2L2, ESCs accumulated heterochromatin and lost their naïve pluripotency. Mad2l2-deficient PGCLCs were locked in their stably repressed chromatin configuration.

Focus Formation in ESCs and Mitomycin-Treated MEFs

Not only MAD2L2, but also other DNA damage response factors accumulated in microfoci in the nuclei of ESCs, although there was no prior induction of DNA damage (Figures 2A–2C) (Banath et al., 2009; Turinetto et al., 2012). Phosphorylation of histone H2AX and accumulation γH2AX foci around the sites of DNA breaks are usually an immediate-early response to DNA damage. In ESCs they were mainly localized, but not necessarily restricted, to areas of low DNA density (Figure 2A). Foci of the E3 ligase RNF168 and of the large adaptor protein 53BP1 were restricted to open chromatin, and their sizes were smaller, quite similar to the MAD2L2 microfoci (Figures 2B and 2C).

ESCs were co-cultured on feeder layers consisting of mitomycin-treated (i.e., DNA damaged) murine embryonic fibroblasts (mMEFs). This allowed a side-by-side comparison of foci formed in response to DNA damage with ESC foci (Figures 2D–2F). Mitomycin C-induced MAD2L2 foci varied widely in size (1–3.5 μm) in the intensity of staining for MAD2L2 and in numbers per nucleus, clearly due to the random nature of DNA damage induction. Focus sizes in ESCs were generally smaller (approximately 0.2–0.3 μm) and the numbers per cell were drastically higher, being homogeneously distributed throughout the open chromatin regions of ESC nuclei, separate from DAPI-stained, condensed heterochromatin (Figures 2D–2F). Thus, the parallel scanning of ESCs and mMEFs revealed an inverse relationship between the level of MAD2L2 and the presence of DAPI-stained heterochromatin. It also demonstrated the principal differences underlying focus formation in response to DNA damage and in ESCs.

Repression of the Dppa3 Locus in the Absence of MAD2L2

We had previously compared the transcriptomes of Mad2l2-deficient and wild-type ESCs, and identified major transcriptional changes (Pirouz et al., 2013). A re-evaluation of the transcriptome data (see Experimental Procedures) revealed that the fourth most significantly down-regulated gene was Dppa3 (Figure 3B). Downregulation of
Dppa3 in the absence of MAD2L2 was confirmed by RT-PCR for ESCs, EpiLCs, and PGCLCs (Figure 3C), and for ESCs also by immunofluorescence (Figure 3A). We used the BVSC cell line to visualize Dppa3 expression by fluorescence of the DPPA3-eCFP fusion protein. DPPA3-positive and -negative ESCs were sorted based on expression of the ECFP reporter, and qPCR confirmed a positive correlation between Dppa3 and Mad2l2 levels. When sorted ESCs with a low level of DPPA3 were recultured in LIF/2i medium, 7% of the cells had upregulated the expression of Dppa3, suggesting that Mad2l2 expression also could recover (Figures S3A–S3C).

ChIP-seq analysis of the Dppa3 locus revealed that its transcriptional repression in Mad2l2-deficient ESCs was associated with the deposition of the repressive histone H3K9me2 in two positions upstream of the transcriptional start site (Figure 3D). The expression of Dppa3 could be rescued by the introduction of a vector encoding a MAD2L2-GFP fusion protein into Mad2l2-deficient ESCs (Figure 3E). In this experiment the reactivation of the Dppa3 gene occurred in the absence of DPPA3 protein. Thus, the initial effect of MAD2L2 was exerted independently of the epigenetic function(s) of DPPA3. Once derepressed, the Dppa3 locus could be transcribed in ESCs, probably under control of the core pluripotency factor PRDM14, which was previously shown to be necessary for Dppa3 expression in PGCLCs (Magnusdottir et al., 2013).

Remarkably, ESC colonies overexpressing GFP-MAD2L2 expressed DPPA3 as visualized by a Dppa3-eCFP transgene uniformly without an indication for heterogeneity (Figure 3F).

Global Increase of DNA Methylation, and Specific Demethylation of Imprinted Loci in Mad2l2-Deficient ESCs
We measured a significant increase of the global levels of 5-methylcytosine in Mad2l2-deficient ESCs compared...
Figure 3. Dppa3 as a Target of MAD2L2

(A) Levels of DPPA3 protein are heterogeneous in a colony of wild-type ESCs (Hayashi et al., 2008), while no DPPA3 is detected in Mad2l2-deficient ESCs. (B) Comparison of transcriptomes from wild-type and Mad2l2-deficient ESCs. The volcano plot displays downregulated genes on the left and upregulated genes on the right. Black dots refer to insignificant changes, and red and green dots to statistically significant up- or downregulated genes, with a log2 fold-change cutoff. The arrow points to Dppa3 as the fourth most strongly decreased gene. (C) Dppa3 mRNA levels in ESCs, EpiLCs, and PGCLCs, in the presence (black) or absence (gray) of Mad2l2. SDs were calculated based on three experimental replicates. (D) Deposition of H3K9me2 is significantly increased in two specific positions upstream of the Dppa3 transcription start site (TSS) in Mad2l2-deficient ESCs. The TSS was taken from the Database of Transcriptional Start Sites (DBTSS, http://dbtss.hgc.jp). Three experimental replicates were performed. (E) RT-PCR analysis demonstrated the downregulation of Dppa3 expression in Mad2l2-deficient ESCs. Expression of Dppa3 mRNA was efficiently rescued after transfection with an expression vector encoding a GFP-DPPA3 fusion protein. SDs were calculated based on three experimental replicates. (F) BVSC ESCs (Ohinata et al., 2008) were transfected with GFP-Mad2l2, resulting in either completely GFP-positive or GFP-negative colonies. Dppa3 expression was visualized by the blue fluorescence of ECFP protein originating from the transgene stably integrated in the endogenous Dppa3 locus. Note the high, uniform expression of ECFP-DPPA3 in the Mad2l2-overexpressing colony, whereas the untransfected colony displayed normal heterogeneity. Three experimental replicates were performed. Scale bars represent 25 μm (A), 25 μm (E), and 50 μm (F). See also Figure S3.
Figure 4. MAD2L2 Regulates DNA Methylation via DPPA3

(A) The low level of 5mC detected by dot blots prepared from ESCs grown in LIF/2i conditions was significantly elevated in mutant ESCs, without, however, reaching the level detected in EpiLCs. This observation was confirmed with three experimental replicates.

(B) 5mC-containing DNA fragments were immunoprecipitated and sequenced. This analysis revealed a global increase of DNA methylation in Mad2l2-deficient ESCs at single-base resolution. Three experimental replicates were performed.

(C) The patterns of DNA methylation were significantly altered in the mutant ESCs. For instance, methylation around the transcription start site was distributed in a single peak in wild-type cells, whereas a three-peak pattern was observed in mutant ESCs. See also class III in Figure S4. Red, green, and blue curves represent the values from three experimental replicates.

(D) Direct comparison of RNA-seq data with the MeDIP data. The volcano plot compares expression of imprinted genes in Mad2l2-deficient ESCs with wild-type ESC; on the right are activated genes, on the left repressed genes. Black dots refer to insignificant changes, while red and green dots refer to significantly up- or downregulated genes with a log2 fold-change cutoff. Note that the top five genes (labeled with asterisks), which were both demethylated and transcriptionally activated in Mad2l2-deficient ESCs, are known imprinted genes.

(E and F) Model for the binding of methyltransferases and interacting proteins near the replication fork. (E) The replication fork in the presence of low MAD2L2 levels as seen, for example, in somatic cells, Mad2l2−/− ESCs, Mad2l2−/− PGCLCs. In the absence of MAD2L2 and DPPA3, H3K9me2 can contribute to the recruitment of DNMT1, UHRF1, EHMT1, and EHMT2. Consequently, the methylation of DNA and (legend continued on next page)
with wild-type cells, although the high level found in EpiLCs was not reached (Figure 4A). Analysis of DNA methylation by methylated DNA immunoprecipitation sequencing (MeDIP-seq) confirmed the observed global difference, and allowed the display of DNA methylation at a single-nucleotide resolution (Figure 4B). These data revealed substantial, genome-wide perturbations of methylation patterns. For example, they demonstrated the presence of three methylation peaks instead of one around transcriptional start sites in mutant ESCs (Figures 4C and S4). Noteworthy, however, is that the Dppa3 gene and its upstream region remained largely unmethylated in both wild-type and mutant ESC. In early embryonic cells several genes maintain their methylation in a widely undermethylated epigenetic landscape, and consequently are transcriptionally repressed (Habibi et al., 2013). DPPA3 was previously shown to play a role in this imprinting of genes (Bortvin et al., 2004; Kang et al., 2013; Nakamura et al., 2007). To study this process in Mad2l2-deficient, and consequently Dppa3-repressing, ESCs, we compared the RNA-seq datasets with the MeDIP-seq data. A total of 221 genes were methylated in wild-type ESCs but were unmethylated and transcriptionally upregulated in the Mad2l2-deficient ESCs (Figure 4D). Of these, the top five (Peg3, Tpm3, Ampd3, Mest, and Nnat) were known imprinted genes (http://www.geneimprint.com). Taken together, the perturbation of DNA methylation in Mad2l2−/− ESCs not only resulted in a general increase of methylation, but also affected imprinting through demethylation of specific loci. Although the Mad2l2-deficient ESCs were shown to be pluripotent and capable of contributing to chimeras (Pirouz et al., 2015), they were not classified as naïvely pluripotent cells with regard to the increased level of DNA methylation.

**DISCUSSION**

The absence of MAD2L2 in mutant ESCs corresponds with the low MAD2L2 levels observed in somatic cells (Figure 4E). Cells with a low level of MAD2L2 have in common that the inhibitory binding of MAD2L2 to EHMT1 and EHMT2 is reduced (Pirouz et al., 2013). Thus, histone H3 can be efficiently methylated on residue K9, and H3K9me2 can then form a docking place for further epigenetic regulators. DNA methyltransferase DNMT1 is recruited together with UHRF1 and EHMT2 at the replication fork, where it will also bind to proliferating cell nuclear antigen, and cooperatively to hemimethylated DNA (Esteve et al., 2006; Liu et al., 2013; Rothbart et al., 2012). Together, these and other interacting factors represent an assembly of both chromatin writers and readers, which maintain and establish a cell-specific chromatin configuration (Liu et al., 2013; Shinkai and Tachibana, 2011). Collectively, the low levels of MAD2L2 and DPPA3 correspond to high levels of repressive histone modifications, substantial DNA methylation, and thus a closed chromatin configuration. In contrast, MAD2L2 levels are high in wild-type ESCs and PGCLCs, downstream of a cascade resembling the DNA damage response pathway (Figures 1A–1I and S2A). In such cells MAD2L2 can directly bind to both EHMT1 and EHMT2, suppress EHMT2 expression, and thus inhibit histone H3K9 and DNA methylation (Pirouz et al., 2013). By antagonizing the formation of H3K9me2, MAD2L2 will derepress the Dppa3 gene. DPPA3 can then displace DNMT1 from UHRF1 at the replication fork, so that the growing strand cannot be methylated (figure 4F; Funaki et al., 2014). In any case, the combined presence of MAD2L2 and DPPA3 in ESCs correlates with their generally low levels of both DNA and histone H3K9 methylation, i.e., a generally open chromatin configuration. In conclusion, the global promotion of open chromatin, and the inhibition of heterochromatin in ESCs and germ cells (PGCLCs and PGCs) depend critically on MAD2L2. The local opening of chromatin observed around DNA breaks may involve MAD2L2 and a similar mechanism (Noon et al., 2010). It is conceivable that the major importance of MAD2L2 lies in its epigenetic functions, be it in a stem or germ cell context, or in response to DNA damage.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

Mad2l2-deficient ESCs carrying an Oct4-GFP allele (Pirouz et al., 2015), and BVSC ESCs, with an insertion of the CFP reading frame into the Dppa3 locus (Ohinata et al., 2008) were cultured in LIF/2i medium (Ying et al., 2008). For PGCLC formation, ESCs were first differentiated for 2 days into EpilCs in the presence of activin and fibroblast growth factor, and subsequently for 6 days in PGCLC differentiation medium as described by Hayashi et al. (2011). See Supplemental Experimental Procedures for further details.

**Flow Cytometry**

Three independent biological replicates of 10,000 cells each were read by an Accuri C6 cytometer (BD Biosciences). See Supplemental Experimental Procedures for further details.
Cell Fractionation
Three independent biological replicates were prepared from 10^8 cells each, and were fractionated into cytoplasmic, nucleoplasmic, and chromatin fractions. See Supplemental Experimental Procedures for further details.

RT-PCR
At least three independent replicates, with two technical replicates each, were performed using the following primer pairs: Gapdh (5’-GTC GTG GAG TCT ACT GGT GTT-3’ and 5’-GAG CCC TTC CAC AAT GCC AAA-3’), Dppa3 (5’-CGT CCT ACA ACC AGA AAC AC-3’ and 5’-CTG CTC AAT CCG AAC AAG TC-3’), and Mad2l2 (5’-GTG GCC TTG AGT CCC TAC AG-3’ and 5’-TCC ACA TCG TTC TTC CCC AG-3’).

Immunocytochemistry
Immunocytochemistry was performed as specified in Supplemental Experimental Procedures. Sections were analyzed by confocal or stimulated emission depletion microscopy.

ChIP-Seq and MeDIP-Seq
ChIP was performed for three independent replicates as described in Supplemental Experimental Procedures (Adli and Bernstein, 2011).

RNA-Seq
Previously reported fastq files (Pirouz et al., 2015) were re-evaluated as specified in the Supplemental Experimental Procedures.

Dot Blot
Dot-blot analysis was performed as described by Hayashi et al. (2011). Three independent replicates of 50 ng DNA were applied to a membrane filter, and the methylation level was detected with an antibody against 5mC (Abcam).

ACCESSION NUMBERS
Sequencing data are available from the European Nucleotide Archive under accession number ENA: PRJEB19344 at http://www.ebi.ac.uk/ena/data/view/PRJEB19344.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, four figures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2017.02.011.

AUTHOR CONTRIBUTIONS
M.K. initiated, led, and managed the project, and wrote the paper. A.R. co-designed the study, performed the majority of experiments and the bioinformatics analyses, and contributed critically to text and figures. M.P. generated the Mad2l2-deficient ES line, and was involved in the early concept of the project. M.d.V. helped with interpretations. D.K. performed stimulated emission depletion microscopy.

ACKNOWLEDGMENTS
The work was supported by the Max Planck Society. M.D.V. is a Helmholtz Association young investigator. We thank M. Saitou (Kyoto University) and M. Boiani (MPI Muenster) for ESC lines, G. Salinas (Goettingen University) for sequencing, and H. Jäckle for his hospitality.

Received: August 30, 2016
Revised: February 13, 2017
Accepted: February 14, 2017
Published: March 16, 2017

REFERENCES
Adli, M., and Bernstein, B.E. (2011). Whole-genome chromatin profiling from limited numbers of cells using nano-ChIP-seq. Nat. Protoc. 6, 1656–1668.
Banath, J.P., Banuelos, C.A., Klokov, D., MacPhail, S.M., Lansdorp, P.M., and Olive, P.L. (2009). Explanation for excessive DNA single-strand breaks and endogenous repair foci in pluripotent mouse embryonic stem cells. Exp. Cell Res. 315, 1505–1520.
Boersma, V., Moatti, N., Segura-Bayona, S., Peuscher, M.H., van der Torre, J., Wevers, B.A., Orthwein, A., Durocher, D., and Jacobs, J.J. (2015). MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5’ end resection. Nature 521, 537–540.
Bortvin, A., Goodheart, M., Liao, M., and Page, D.C. (2004). Dppa3/Pgc7/stella is a maternal factor and is not required for germ cell specification in mice. BMC Dev. Biol. 4, 2.
Esteve, P.O., Chin, H.G., Smallwood, A., Feehery, G.R., Gangisetty, O., Karpf, A.R., Carey, M.F., and Pradhan, S. (2006). Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. Genes Dev. 20, 3089–3103.
Funaki, S., Nakamura, T., Nakatani, T., Umehara, H., Nakashima, H., and Nakano, T. (2014). Inhibition of maintenance DNA methylation by Stella. Biochem. Biophys. Res. Commun. 453, 455–460.
Gaspar-Maia, A., Alajem, A., Meshorer, E., and Ramalho-Santos, M. (2011). Open chromatin in pluripotency and reprogramming. Nat. Rev. Mol. Cell Biol. 12, 36–47.
Habibi, E., Brinkman, A.B., Arand, J., Kroeze, L.I., Kerstens, H.H., Matarese, E., Lepikhov, K., Gut, M., Brun-Heath, I., Hubner, N.C., et al. (2013). Whole-genome bisulfite sequencing of two distinct interconvertible DNA methylomes of mouse embryonic stem cells. Cell Stem Cell 13, 360–369.
Hayashi, K., Lopes, S.M., Tang, E., and Surani, M.A. (2008). Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. Cell Stem Cell 3, 391–401.
Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S., and Saitou, M. (2011). Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 146, 519–532.
Kang, J., Kalantry, S., and Rao, A. (2013). PGC7, H3K9me2 and Tet3: regulators of DNA methylation in zygotes. Cell Res. 23, 6–9.
Liu, X., Gao, Q., Li, P., Zhao, Q., Zhang, J., Li, J., Koseki, H., and Wong, J. (2013). UHRF1 targets DNMT1 for DNA methylation.
through cooperative binding of hemi-methylated DNA and methylated H3K9. Nat. Commun. 4, 1563.
Magnusdottir, E., Dietmann, S., Murakami, K., Gunesdogan, U., Tang, F., Bao, S., Diamanti, E., Lao, K., Gottgens, B., and Azim Surani, M. (2013). A tripartite transcription factor network regulates primordial germ cell specification in mice. Nat. Cell Biol. 15, 905–915.
Meshorer, E., and Misteli, T. (2006). Chromatin in pluripotent embryonic stem cells and differentiation. Nat. Rev. Mol. Cell Biol. 7, 540–546.
Nakamura, T., Arai, Y., Umehara, H., Masuhara, M., Kimura, T., Taniguchi, H., Sekimoto, T., Ikawa, M., Yoneda, Y., Okabe, M., et al. (2007). PGC7/Stella protects against DNA demethylation in early embryogenesis. Nat. Cell Biol. 9, 64–71.
Noon, A.T., Shibata, A., Rief, N., Lobrich, M., Stewart, G.S., Jeggo, P.A., and Goodarzi, A.A. (2010). 53BP1-dependent robust localized KAP-1 phosphorylation is essential for heterochromatic DNA double-strand break repair. Nat. Cell Biol. 12, 177–184.
Ohinata, Y., Sano, M., Shigeta, M., Yamanaka, K., and Saitou, M. (2008). A comprehensive, non-invasive visualization of primordial germ cell development in mice by the Prdm1-mVenus and Dppa3-ECFP double transgenic reporter. Reproduction 136, 503–514.
Payer, B., Saitou, M., Barton, S.C., Thresher, R., Dixon, J.P., Zahn, D., Colledge, W.H., Carlton, M.B., Nakano, T., and Surani, M.A. (2003). Stella is a maternal effect gene required for normal early development in mice. Curr. Biol. 13, 2110–2117.
Pirouz, M., Pilarski, S., and Kessel, M. (2013). A critical function of Mad2L2 in primordial germ cell development of mice. PLoS Genet. 9, e1003712.
Pirouz, M., Rahjouei, A., Shamsi, E., Eckermann, K.N., Salinas-Riester, G., Pommerenke, C., and Kessel, M. (2015). Destabilization of pluripotency in the absence of Mad2L2. Cell Cycle 14, 1596–1610.
Rothbart, S.B., Krajewski, K., Nady, N., Tempel, W., Xue, S., Badeaux, A.I., Barsyte-Lovejoy, D., Martinez, J.Y., Bedford, M.T., Fuchs, S.M., et al. (2012). Association of UHRF1 with methylated H3K9 directs the maintenance of DNA methylation. Nat. Struct. Mol. Biol. 19, 1155–1160.
Shinkai, Y., and Tachibana, M. (2011). H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev. 25, 781–788.
Singer, Z.S., Yong, J., Tischler, J., Hackett, J.A., Altinok, A., Surani, M.A., Cai, L., and Elowitz, M.B. (2014). Dynamic heterogeneity and DNA methylation in embryonic stem cells. Mol. Cell 55, 319–331.
Turinnetto, V., Orlando, L., Sanchez-Ripoll, Y., Kumpfmuller, B., Storm, M.P., Porcedda, P., Minieri, V., Saviozzi, S., Accomasso, L., Cibarico Rocchietti, E., et al. (2012). High basal gammaH2AX levels sustain self-renewal of mouse embryonic and induced pluripotent stem cells. Stem Cells 30, 1414–1423.
Willig, K.I., Rizzoli, S.O., Westphal, V., Jahn, R., and Hell, S.W. (2006). STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. Nature 440, 935–939.
Xu, G., Chapman, J.R., Brandsma, I., Yuan, J., Mistrik, M., Bouwman, P., Barkova, J., Gogola, E., Warmerdam, D., Barazas, M., et al. (2015a). REV7 counteracts DNA double-strand break resection and affects PARP inhibition. Nature 521, 541–544.
Xu, X., Smorag, L., Nakamura, T., Kimura, T., Dressel, R., Fitzner, A., Tan, X., Linke, M., Zechnier, U., Engel, W., et al. (2015b). Dppa3 expression is critical for generation of fully reprogrammed iPS cells and maintenance of Dlk1-Dio3 imprinting. Nat. Commun. 6, 6008.
Ying, Q.L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P., and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. Nature 453, 519–523.