Genome-wide DNA methylation dynamics during epigenetic reprogramming in the porcine germline

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Abstract

Background: Prior work in mice has shown that some retrotransposed elements remain substantially methylated during DNA methylation reprogramming of germ cells. In the pig, however, information about this process is scarce. The present study was designed to examine the methylation profiles of porcine germ cells during the time course of epigenetic reprogramming.

Results: Sows were artificially inseminated, and their fetuses were collected 28, 32, 36, 39, and 42 days later. At each time point, genital ridges were dissected from the mesonephros and germ cells were isolated through magnetic-activated cell sorting using an anti-SSEA-1 antibody, and recovered germ cells were subjected to whole-genome bisulphite sequencing. Methylation levels were quantified using SeqMonk software by performing an unbiased analysis, and persistently methylated regions (PMRs) in each sex were determined to extract those regions showing 50% or more methylation. Most genomic elements underwent a dramatic loss of methylation from day 28 to day 36, when the lowest levels were shown. By day 42, there was evidence for the initiation of genomic re-methylation. We identified a total of 1456 and 1122 PMRs in male and female germ cells, respectively, and large numbers of transposable elements (SINEs, LINEs, and LTRs) were found to be located within these PMRs. Twenty-one percent of the introns located in these PMRs were found to be the first introns of a gene, suggesting their regulatory role in the expression of these genes. Interestingly, most of the identified PMRs were demethylated at the blastocyst stage.

Conclusions: Our findings indicate that methylation reprogramming in pig germ cells follows the general dynamics shown in mice and human, unveiling genomic elements that behave differently between male and female germ cells.

Keywords: Embryo, Primordial germ cells, Methylation reprogramming, Whole-genome bisulphite sequencing

Background

Mammalian genomes undergo epigenetic reprogramming, which mostly involves the reprogramming of histone modifications and the erasure and re-establishment of DNA methylation [51 Tmat

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be made with caution. Recent studies have also examined DNA de-methylation during the reprogramming of germ cells in humans [5–8]. However, re-methylation in human germ cells has not been explored, and di erences were detected with respect to mouse PGCs including their mitotic behaviour during the de-methylated period.

e pig is a broadly used model for human, as pigs are evolutionarily closer to human than mice [9, 10] and both species share various physiologic and anatomic characteristics [11]. Some studies have identified key aspects of porcine germ cell methylation and development [12, 13], yet more extensive studies are necessary to fully understand the dynamics of epigenetic reprogramming.

is information will help understand the mechanism of reprogramming of gonadal germ cells in mammals.

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window containing a minimum of 20 observations per feature, yielding a total of 327,583 tiles. We found that on day 28, overall DNA methylation, as evaluated from the median methylation of 100-CpG tiles, was 15.38% in male germ cells and 15.85% in female germ cells. Given that the methylation level in somatic cells of the pig fetus on day 28 has been reported at around 75% [25], it seems the main wave of DNA demethylation occurred in the germ cells before day 28 (Fig. 2a). We observed that in both male and female germ cells, median meth-

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shown), suggesting that pig gonad germ cells feature only marginal DNA methylation levels of non-CpG sites.

Methylation reprogramming of functional genomic elements in porcine germ cells

We further analysed the methylation dynamics of different functional genomic features. A general view of the methylation profile of these elements is shown in Fig. 3a, and their means is represented in Fig. 3b. A predominantly low level of methylation was observed in all the samples, with few tiles having a high methylation level (>50%). Remarkably, the same clear pattern that we observed in the averaged methylation of CpG sites could be seen when looking at the methylation of functional genomic elements, for which a drop in the methylation levels from D28 to the lowest levels on D36, and then recovering it on D42 was observed. is general trend can be seen in all elements analysed in both sexes, although the demethylation trough was more pronounced in male germ cells. is could indicate differences in the start of re-methylation between male and female germ cells, again being cautions due to the low sample size of D28 and D42. In the case of CGI-containing promoters, the demethylation at D36 was even more marked: methylation fell to 8.54% in the case of males and 10.04% in females, whereas in promoters not located within a CGI the reduction of methylation was attenuated (Fig. 3b, Ad-57 4w 9.8 56.692902t720J 0 5-5.98212894.455999851 0 Td (3)Tj 0 0 0 rgMC 3I(e of Tw 0 s3).5(.9(tw.5 0

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We analysed separately the methylome dynamics of the X-chromosome, evaluating the methylation profiles of promoters containing CGIs of male and female germ cells. We observed that, in male germ cells, those promoters follow the general de- and re-methylation dynamics described above, whereas in female germ cells we detect higher methylation levels on D28, and we do not observe re-methylation after D36 (Additional file 4A, left panel). Most promoters showing around 20% of methylation at day 28 in female germ cells are hypomethylated from D36 onwards, suggesting that methylation of the inactive X-chromosome begins in later stages (Additional file 4A, right panel).
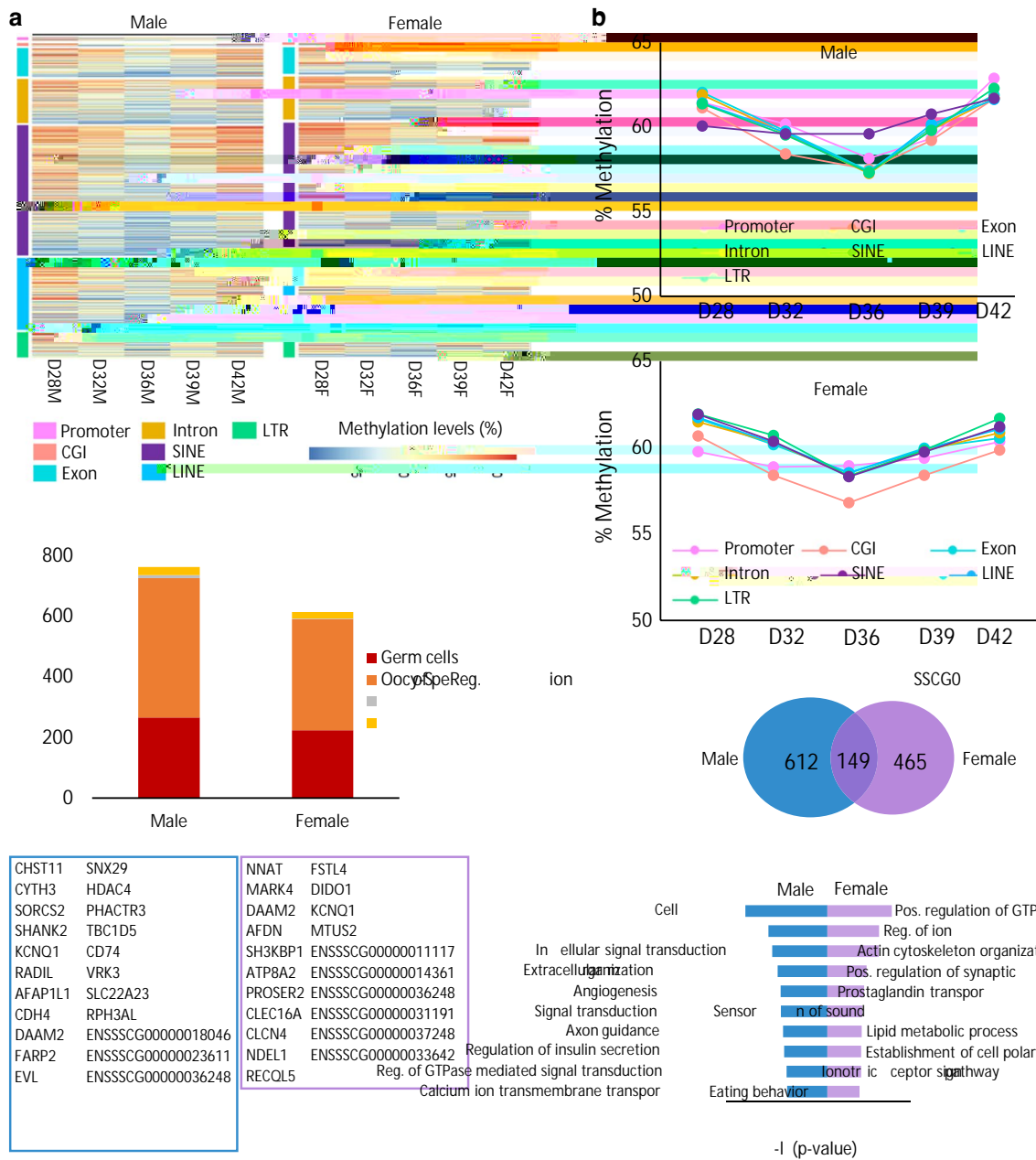
We also observed a di erent pattern of methylation between male and female germ cells in some genes involved in meiosis, by evaluating the averaged methylation levels of promoters of 70 genes involved in meiosis. In the case of male germ cells, we observed mean methylation levels around 22% in all days analysed. For female germ cells, we observed a di erent pattern: first, the mean methylation levels of the meiotic genes in germ cells are lower at all days analysed, and they drop from 15% at day 28 to ∼ 9% from day 36 onwards (Additional file 4B, right panel). Moreover, we detected a set of genes that are highly methylated in males and demethylated in females, including *TRIP13* and *CDC25,* that belong to the ontology term of female meiosis I, *TR ted s*

spindle organization, chromosome alignment, and cell cycle progression in mouse oocytes [40] (Additional file 4B, left panel).

Methylome dynamics of major transposable elements in porcine germ cells

Transposable elements comprise about half of the genome in mammals, and over 80% of pig protein-coding and lncRNA genes overlap with retrotransposon insertions [41]; thus, their regulation in terms of DNA methylation reprogramming is important to understand. We examined methylation patterns of the three major types of transposable elements: SINEs, LINEs, and LTRs. A

a

Male   Female

Promoter   Intron   LTR
CGI   SINE
Exon   LINE

Methylation levels (%)

Germ cells
Oocyte/Sperm
Reg.

b

% Methylation

Male

Promoter   CGI   Exon
Intron   SINE   LINE
LTR

D28   D32   D36   D39   D42

% Methylation

Female

Promoter   CGI   Exon
Intron   SINE   LINE
LTR

D28   D32   D36   D39   D42

SSCG0

Male   612   149   465   Female

CHST11	SNX29
CYTH3	HDAC4
SORCS2	PHACTR3
SHANK2	TBC1D5
KCNQ1	CD74
RADIL	VRK3
AFAP1L1	SLC22A23
CDH4	RPH3AL
DAAM2	ENSSSCG00000018046
FARP2	ENSSSCG00000023611
EVL	ENSSSCG00000036248

NNAT	FSTL4
MARK4	DIDO1
DAAM2	KCNQ1
AFDN	MTUS2
SH3KBP1	ENSSSCG00000011117
ATP8A2	ENSSSCG00000014361
PROSER2	ENSSSCG00000036248
CLEC16A	ENSSSCG00000031191
CLCN4	ENSSSCG00000037248
NDEL1	ENSSSCG00000033642
RECQL5	

Male   Female

Cell   Pos. regulation of GTPase activity
Reg. of ion   transpor
Intracellular signal transduction   Actin cytoskeleton organization
Extracellular organization   Pos. regulation of synaptic   on
Angiogenesis   Prostaglandin transpor
Signal transduction   Sensor   n of sound
Axon guidance   Lipid metabolic process
Regulation of insulin secretion   Establishment of cell polarity
Reg. of GTPase mediated signal transduction   Ionotr ic   ceptor sign pathway
Calcium ion transmembrane transpor   Eating behavior

-l (p-value)

methylation reprogramming has been described as the main impediment to intergenerational epigenetic inheritance [47]. We identified genomic features located within those regions, which accordingly were able to avoid methylation erasure, as being potentially involved in this process, as described in mouse and human germ cells [6, 7, 22, 48]. To our knowledge, this is the first description of genome regions resistant to methylation erasure in the pig, and we believe it to be a good starting point for the investigation of the potential for epigenetic inheritance in livestock species. We identified 1,456 and 1,122 regions remaining persistently methylated (methylation at least 50%) in male and female germ cells, respectively, of which the majority were sex-specific. However, fewer than 3% of the PMRs also remain highly methylated in pig blastocysts, indicating that their methylation is erased during epigenetic reprogramming in the preimplantation embryo, thus making them unlikely mediators of intergenerational epigenetic inheritance.

Remarkably, a significant percentage of introns found to escape de-methylation were the first introns of a gene ($\sim 21\%$). Indeed, these elements have special functional roles, such as regulating the correct cytoplasmic localisation of some mRNAs [49], and inverse correlation has been described between DNA methylation of the first intron and gene expression across tissues [50]. Persistently methylated transposable elements (SINEs, LINEs, and LTRsb

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was incubated for 3 min at 37ºC. TrypLE Express was then blocked with 3 ml of fetal calf serum (FCS) and the solution filtered through a cell strainer (40 μm) and cen-

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containing at least 6 CpG sites in each stage (D28, D32, D36, D39, and D42), and selected those regions showing a methylation value of 50% or above in all samples, yielding a total of 1,456 PMRs in the male germ cells and 1,122 PMRs in the female germ cells.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-021-01003-x.

Additional le 1: Summary of sequencing statistics of each sample.

Additional le 2: Mean methylation levels of genomic elements.

Additional le 3: General view of the imprinted gene *RASGRF1*, demethylation resistant in germ cells. Blue and red dots represent methylation reads. The lower panel shows the methylation levels detected in the di erentially methylated region (DMR) in all samples analysed.

Additional le 4: (A) X-chromosome methylome dynamics. The left panel shows a heatmap representing levels of methylation of X chromosome promoters, each line corresponding to a single feature. High methylation levels are represented in red, and low methylation levels are shown in blue. To the right, line graph representing the mean level of methylation of the X-chromosome promoters on each sex and day. (B) Methylation dynamics of 70 meiosis-related genes. The left panel shows a heatmap representing levels of methylation of genes involved in meiosis, each line corresponding to a single feature. High methylation levels are represented in red, and low methylation levels are shown in blue. Clusters showing di erent patterns of methylation between male and female are zoomed in. To the right, line graph representing the mean level of methylation of the meiosis-related genes analysed on each sex and day.

Additional le 5: Genomic elements reported for male PMRs including their levels of methylation in each sample and their overlap with blastocyst and oocyte-sperm PMRs. The following tabs are included: (A) Promoters, (B) CGIs, (C) Exons, (D) Introns, (E) SINEs, (F) LINEs, (G) LTRs, and (H) Genes. Tab (D) includes information about intron position, tabs (E), (F), and (G) include the element subtype, and tab (H) includes the possible coincidence of a feature in female PMRs.

Additional le 6: Genomic elements included in female PMRs, including their levels of methylation in each sample and their overlap with blastocyst and oocyte-sperm PMRs. The following tabs are included: (A) Promoters, (B) CGIs, (C) Exons, (D) Introns, (E) SINEs, (F) LINEs, (G) LTRs, and (H) Genes. Tab (D) includes information about the position of the intron, tabs (E), (F), and (G) include the subtype of the element, and tab (H) includes the possible coincidence of a feature in male PMRs.

Additional le 7: Summary of the elements included in common PMRs between germ cells (male and female separately) and blastocyst (data from [26]) and sperm-oocyte (data from [27]).

Additional le 8: Summary of the genes identified as persistently methylated in male and female germ cells di erentially expressed in mice and bovine embryos during sex determination or coding for di erent isoforms depending on the sex.

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its additional information files]. Bisulphite-sequencing data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-9326.

Ethics approval
Animal experiments were performed following the recommendations of the European Community Council Directive 2010/63/EU guidelines. Experiments were approved by the Committee on the Ethics of Animal Experiments of the INIA (permit number CEEA 2012/021).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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