Developmental Biology 361 (2012) 403–411

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Antisense noncoding RNA promoter regulates the timing of *de novo* methylation of an imprinting control region

Natalia Guseva [a,1], Tanmoy Mondal [a,1], Chandrasekhar Kanduri [a,b,*]

[a] Uppsala SciLife Laboratory, Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Dag HammarskjöldsVäg 20, S75185, Uppsala University, Uppsala, Sweden
[b] Department of Medical and Clinical genetics, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Gothenberg, Sweden

ARTICLE INFO

Article history:
Received for publication 19 August 2011
Revised 24 October 2011
Accepted 8 November 2011
Available online 15 November 2011

Keywords:
DNA methylation
Chromatin
Epigenetics
Histone modifications
Differentially methylated regions
Genomic imprinting

ABSTRACT

Epigenetic marks at cis acting imprinting control regions (ICRs) regulate parent of origin-specific expression of multiple genes in imprinted gene clusters. Epigenetic marks are acquired during gametogenesis and maintained faithfully thereafter. However, the mechanism by which differential epigenetic marks are established and maintained at ICRs is currently unclear. By using *Kcnq1* ICR as a model system, we have investigated the functional role of genetic signatures in the acquisition and maintenance of epigenetic marks. *Kcnq1* ICR is methylated on the maternal chromosome but remains unmethylated on the paternal chromosome. Here, we show that a paternal allele of *Kcnq1* ICR lacking the *Kcnq1ot1* promoter remains unmethylated during spermatogenesis; however, it becomes methylated specifically during pre-implantation development. Analysis of the chromatin structure at the paternal ICR in spermatogenic cells and in E13.5 embryonic tissues revealed that the ICRs of both wild type and mutant mice are enriched with H3K4me2 in spermatiogenic cells of the testicular compartment, but the mutant ICR lost H3K4me2 specifically in epididymal sperm and an increase in repressive marks was observed in embryonic tissues. Interestingly, we also detected a decrease in nucleosomal histone levels at the mutant ICR in comparison to the wild-type ICR in epididymal sperm. Taken together, these observations suggest that the *Kcnq1ot1* promoter plays a critical role in establishing an epigenetic memory in the male germline by ensuring that the paternal allele remains in an unmethylated state during pre-implantation development.

Introduction

DNA methylation is an important epigenetic modification that regulates crucial biological functions such as genomic imprinting, transposon silencing and chromosomal stability. DNA methylation is mediated by the cellular DNA methylation machinery, comprising Dnmt1, Dnmt3a, Dnmt3b and Dnmt3L. DNA methylation is a dynamic process during early embryonic development and undergoes parent and lineage dependent genome-wide changes. Between 8 dpc and 13 dpc of embryonic development, genome-wide methylation patterns, including parental-specific methylation patterns, are erased in developing primordial germ cells. New methylation patterns are established in the later part of germ cell development according to the sex of the parent. Upon fertilization, most of the genome is epigenetically reprogrammed (Morgan et al., 2005; Stoger et al., 1993; Tremblay et al., 1995). At this time, the paternal genome is actively demethylated within the first few hours of fertilization, while the maternal genome is demethylated passively, with every new cell division (Mayer et al., 2000; Oswald et al., 2000; Rougier et al., 1998). This genome-wide demethylation is followed by a genome-wide *de novo* methylation event, which occurs between the pre-implantation morula and the post-implantation gastrula stage (Weaver et al., 2009). Although genome-wide *de novo* methylation and demethylation events occurring at different developmental stages have been relatively well investigated, the mechanisms underlying these events have not been studied thoroughly.

Studies on a subset of differentially methylated ICRs, which dictate the expression of imprinted gene clusters, have provided greater insights into the dynamics of methylation and demethylation. Genomic imprinting is an epigenetic phenomenon where imprinted genes are expressed monoallelically in a parent-of-origin-specific manner (Reik and Walter, 2001; Tilghman, 1999). Thus far, more than 100 imprinted genes have been identified and the majority of them are organized in clusters, where they are regulated by 2 to 4 kb long differentially methylated ICRs. ICRs are CpG rich regions characterized by differential methylation and histone modifications. The differential epigenetic marks at ICRs are attained during female or male germ cell development and are maintained faithfully after fertilization in all somatic lineages during the rest of development. In every new reproductive cycle, epigenetic marks at ICRs are

* Corresponding author at: Uppsala SciLife Laboratory, Department of Genetics and Pathology, Rudbeck Laboratory, Dag HammarskjöldsVäg 20, S75185, Uppsala University, Uppsala, Sweden.
E-mail address: kanduri.chandrasekhar@igp.uu.se (C. Kanduri).
[1] These authors have contributed equally to this work.

0012-1606/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2011.11.005

erased and are re-established. There are about 21–22 ICRs that have been shown to attain epigenetic modifications during germ cell development and are termed as germline ICRs (Arnaud, 2010). Interestingly, certain CpG rich regions within the imprinted cluster attain differential methylation marks during the pre-/post-implantation *de novo* methylation event and, hence, are known as somatic differentially methylated regions (somatic DMRs). Unlike the germline ICRs, which control the imprinting of multiple genes in an imprinted cluster, the somatic ICRs often regulate the imprinting of an overlapping gene (Mohammad et al., 2008). However, it is unclear what dictates different developmental points for methylating somatic DMRs and germline ICRs.

The majority of germline ICRs (including *Kcnq1*, *Snrpn*, *Mest* and *Peg3*) are methylated in oocytes prior to ovulation, and only four out of the 21–22 germline ICRs are methylated in male germ cells (*H19/Igf2*, *Dlk1/Gtl2*, *Rasgrf1* and *Zdbf2*) between mitotic arrest and birth (Bartolomei, 2009; Hiura et al., 2006, 2010; Li et al., 2004; Lucifero et al., 2004), indicating that germline-specific mechanisms maintain differential epigenetic marks at ICRs. Dnmt3a/3L and/or Dnmt3b have been implicated in the acquisition of methylation imprints at ICRs in the maternal and paternal germlines. However, it is currently unclear how the differential methylation status of ICRs is established and maintained during germline and somatic differentiation. In addition to DNA methylation, germline ICRs also exhibit allele-specific histone modifications, and several studies have proposed a mechanistic link between DNA methylation and histone modifications (Dindot et al., 2009; Fournier et al., 2002; Gregory et al., 2001; Hu et al., 2000; Lucifero et al., 2004). Indeed, this mechanistic relationship has been probed in a recent report, where Dnmt3L−/− female mice, which lacked DNA methylation, showed a loss of the allele-specific repressive histone modifications H3K9me3, H4K20me3 and H2A/H4R3me2 in many maternally methylated germline ICRs, including the *Kcnq1* ICR (Henckel et al., 2009). Moreover, Dnmt3L has been shown to induce *de novo* DNA methylation by interacting with histone H3 tails that are unmethylated at Lys4 (Ooi et al., 2007). Although the latter data establishes a functional link between the two types of epigenetic marks, it is unclear whether DNA methylation or histone modifications act as the primary epigenetic mark. The *Kcnq1* ICR (KvDMR or IC2), located in intron 10 of the *Kcnq1* gene, is a maternally methylated ICR (Fitzpatrick et al., 2002). The ICR on the paternal allele is unmethylated and harbors an actively transcribing promoter for a long noncoding RNA *Kcnq1ot1*, which is implicated in the transcriptional silencing of 8–10 protein coding RNA genes, which are located on either side of the promoter. Thus, the differential methylation of the *Kcnq1* ICR determines the parent of origin-specific expression profiles of genes within the *Kcnq1* domain (Mohammad et al., 2008; Shin et al., 2008). Changes in CpG methylation at the *Kcnq1* ICR have been linked with Beckwith–Wiedemann Syndrome (BWS). About 50% of sporadic BWS patients exhibit hypomethylation of the maternal *Kcnq1* ICR (Weksberg et al., 2009). BWS is a pediatric disorder of growth regulation that often results in somatic overgrowth and a predisposition to embryonal malignancies.

In this study, we attempt to functionally dissect the mechanisms that protect the paternal allele of the *Kcnq1* ICR from *de novo* methylation at different developmental stages. We show that the *Kcnq1ot1* promoter region regulates the differential methylation of the *Kcnq1* ICR. Like the wild-type ICR, the paternal ICR, lacking the 244 bp *Kcnq1ot1* promoter, remains unmethylated during spermatogenesis but becomes fully methylated by genome-wide methylation during pre-implantation development. Analysis of the chromatin structure of the wild-type (WT) and mutant ICRs during spermatogenesis revealed that both mutant and wild-type ICRs are enriched with H3K4me2 methylation in testicular spermatocytes, but the H3K4me2 mark is specifically lost at the mutant ICR during spermiogenesis. Moreover, the WT ICR, but not the mutant ICR, retained nucleosomal histones during spermiogenesis, indicating that the *Kcnq1ot1* promoter region contains the information for retaining nucleosomes during spermiogenesis. Collectively, these observations suggest that the interplay between genetic and chromatin signatures determines the differential methylation of the *Kcnq1* ICR, and in the absence of the *Kcnq1ot1* promoter sequence, the ICR on the paternal chromosome succumbs to *de novo* methylation in the early embryo.

Results

The Kcnq1ot1 promoter is crucial for maintaining differential methylation of the ICR at early embryonic stages

The *Kcnq1* ICR, a master regulator of imprinting in the one megabase *Kcnq1* domain, is methylated on the maternal chromosome but remains unmethylated on the paternal chromosome. The *Kcnq1* ICR maps to intron 10 of the *Kcnq1* gene and harbors a promoter for noncoding RNA, *Kcnq1ot1*. *Kcnq1ot1* is expressed only from the paternal unmethylated *Kcnq1* ICR. The *Kcnq1* ICR has two CpG islands: one (CpG1) maps to the *Kcnq1ot1* promoter, and the other (CpG2) maps to the 890 bp *Kcnq1ot1* silencing domain (SD), which is critical for transcriptional silencing of ubiquitously imprinted genes (Mohammad et al., 2010). To date, it is not clear whether there are any specific sequences within the *Kcnq1* ICR that control the establishment and/or maintenance of differential methylation marks at the ICR. Previously, several deletions were created within the *Kcnq1* ICR, and the effect of these deletions on the differential methylation of the ICR was investigated (Fig. 1A). Surprisingly, none of the deletions had any effect on the differential methylation of the ICR. For example, a 657 bp deletion, which is immediately downstream of the *Kcnq1ot1* promoter, is part of the CpG1 and harbors highly conserved MD1 repeats (Δrep mouse), had no effect on the differential methylation of the ICR (Mancini-Dinardo et al., 2006). Truncation of the *Kcnq1ot1* RNA by inserting a transcription termination signal at 1.5 kb (Mancini-Dinardo et al., 2006) or at 2.6 kb (Shin et al., 2008) had no effect on the differential methylation of the *Kcnq1* ICR, indicating that the full length RNA has no role in the maintenance of differential methylation at the *Kcnq1* ICR. Another deletion, which covers the CpG2 region and includes the 890 bp SD, also had no effect on CpG methylation of the *Kcnq1* ICR (Mohammad et al., 2010). Since the *Kcnq1ot1* RNA promoter is another important functional element within the *Kcnq1* ICR, we investigated the effect of deleting the *Kcnq1ot1* promoter sequence on the differential methylation of the *Kcnq1* ICR. To test this, we took advantage of the previously reported Δprom knockout mouse, where a 244 bp fragment containing three tandemly organized CCAAT elements was deleted from the ICR (Mancini-Dinardo et al., 2006). First, we investigated the effect of this small deletion, comprising crucial cis acting elements of the *Kcnq1ot1* promoter, on the differential methylation of the CpG1 region of the *Kcnq1* ICR in E13.5 fetal liver tissues. We found that the CpG1 region of the ICR was methylated on both parental alleles when the ICR carrying the promoter deletion was inherited paternally (Fig. 1B), whereas upon maternal inheritance, the deletion had no effect on the methylation status of the *Kcnq1* ICR (Fig. 1C), suggesting that the antisense promoter sequences play a critical role in the maintenance of differential methylation at the *Kcnq1* ICR. To address whether the gain of methylation at the *Kcnq1* ICR upon the promoter deletion is specific to the CpG1 region, we also analyzed the CpG2 region and found a similar gain of methylation, indicating that the entire ICR becomes methylated upon promoter deletion (Fig. S1A, B).

We next investigated the developmental window period in which the *Kcnq1* ICR with the promoter deletion becomes methylated. In WT cells, the *Kcnq1* ICR acquires methylation during oogenesis, while it remains unmethylated during spermatogenesis (Fig. 2A and B). We found that the ICR lacking the *Kcnq1ot1* promoter maintained the