Essays in Biochemistry (2019) **63** 773–783
https://doi.org/10.1042/EBC20190035

Review Article

DNA methylation in disease: Immunodeficiency, Centromeric instability, Facial anomalies syndrome

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DNA methylation is an epigenetic modification essential for normal mammalian development. Initially associated with gene silencing, more diverse roles for DNA methylation in the regulation of gene expression patterns are increasingly being recognized. Some of these insights come from studying the function of genes that are mutated in human diseases characterized by abnormal DNA methylation landscapes. The first disorder to be associated with congenital defects in DNA methylation was Immunodeficiency, Centromeric instability, Facial anomalies syndrome (ICF). The hallmark of this syndrome is hypomethylation of pericentromeric satellite repeats, with mutations in four genes: *DNMT3B*, *ZBTB24*, *CDCA7* and *HELLS*, being linked to the disease. Here, we discuss recent progress in understanding the molecular interactions between these genes and consider current evidence for how aberrant DNA methylation may contribute to the abnormal phenotype present in ICF syndrome patients.

DNA methylation as a regulatory epigenetic mark

DNA methylation, which generally refers to the direct chemical modification of DNA, was discovered more than 80 years ago [1] and is now one of the most studied and best characterized epigenetic modification. In the late 1970s, a role for DNA methylation in the regulation of gene expression was first demonstrated [2,3], and today its critical function in diverse biological processes, such as transcriptional regulation, silencing of transposable elements, genomic imprinting and X-chromosome inactivation, is well established [4]. Not surprisingly, aberrant DNA methylation is a feature of many different types of cancer [5], and genetic defects in components of the DNA methylation machinery have been linked to human congenital disease [6].

In mammals, DNA methylation predominantly occurs on cytosine bases in the symmetrical CpG dinucleotide context, which enables the post-replicative maintenance of DNA methylation patterns. Specifically, the fifth carbon of the cytosine pyrimidine ring is covalently modified by the addition of a methyl group [6]. DNA methylation is widespread in mammals, and approximately 70–80% of all CpGs are methylated. Depending on the genomic context in which it is placed, DNA methylation can be interpreted differently and play diverse regulatory roles. For example, transposable elements, which are dispersed throughout the genome and account for approximately 40% of the mammalian genome [7], are highly methylated sequences [8]. In somatic cells, DNA methylation is required to suppress transposon expression and mobility [8], thereby protecting the genome from deleterious effects of these elements which when mobilized, can cause genome instability [9]. In contrast, CpG islands (CGI), which are CpG-rich sequences often found in the vicinity of promoters, are usually devoid of DNA methylation [10]. Promoter DNA methylation correlates with gene silencing and typically occurs in a tissue- or developmental stage-specific manner [11–13]. Historically, DNA methylation has been associated with gene repression, but it is now known that DNA methylation can also be associated with active transcription, when found across gene bodies

Received: 20 August 2019
Revised: 01 October 2019
Accepted: 02 October 2019

Version of Record published:
14 November 2019

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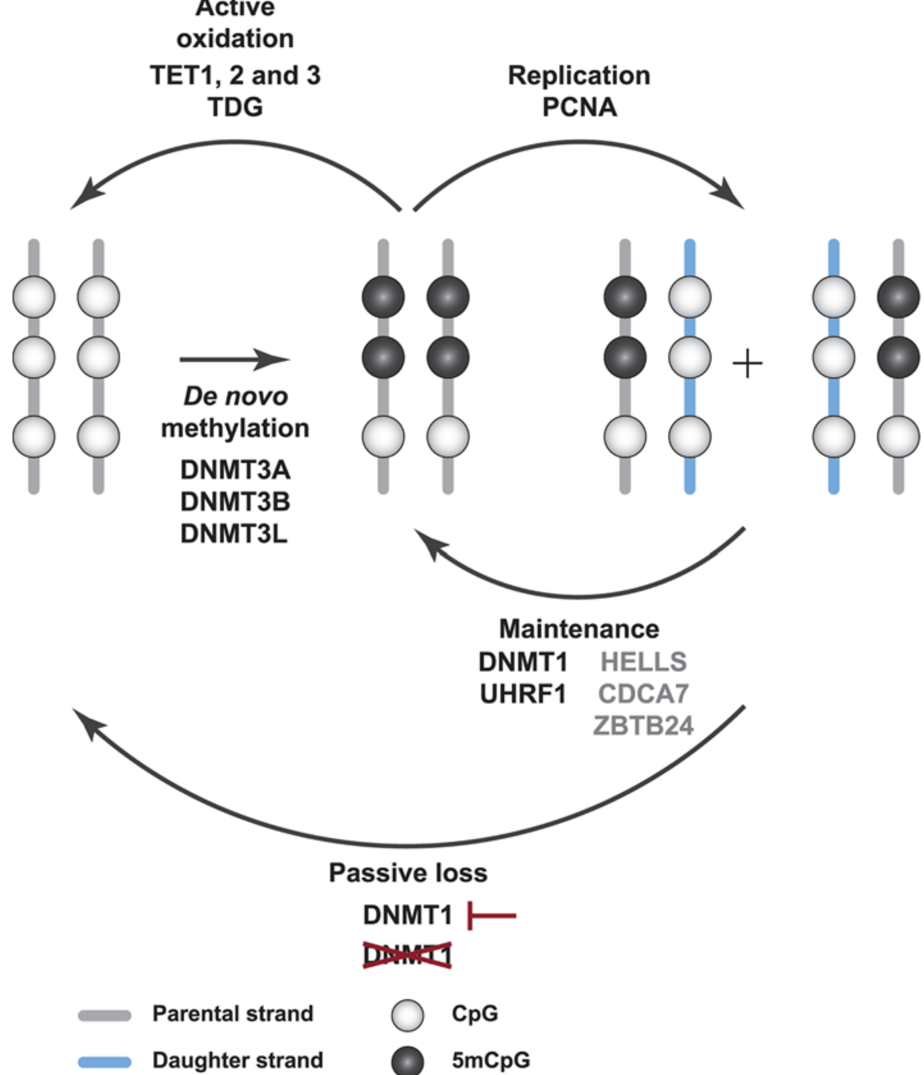

Figure 1. Model of functions of the DNA methylation machinery in establishment and maintenance of DNA methylation patterns

During early embryonic development and gametogenesis, DNA methylation is established by the *de novo* DNA methyltransferases DNMT3A and DNMT3B, together with cofactors such as DNMT3L. After every cell cycle, DNMT1 maintains methylation patterns in daughter cells. DNMT1 recognizes replication foci and hemi-methylated DNA with the help of PCNA and UHRF1, respectively. HELLS, CDCA7 and ZBTB24 (gray) contribute to DNA methylation maintenance at intergenic regions and repetitive elements. DNA methylation can be passively removed, for example, through the absence or inhibition of DNMT1. Active demethylation occurs through the oxidizing activity of TET enzymes. Abbreviations: CDCA7, cell division cycle associated 7; HELLS, helicase lymphoid specific; TET, ten-eleven translocation; ZBTB24, zinc-finger and BTB domain-containing 24.

[4]. Gene body methylation has been suggested to prevent transcription from intragenic/cryptic promoters [14], to play a role in the regulation of splicing [15–17] and to function in transcript elongation [18]. Nevertheless, further studies are required to fully understand the implications of heavily methylated gene bodies.

The DNA methylation machinery

DNA methylation patterns are established by the *de novo* methyltransferases, DNA methyltransferase 3 alpha (DNMT3A) and DNA methyltransferase 3 beta (DNMT3B) during early embryonic and germline development (Figure 1) [19,20]. The catalytic domains of DNMT3s lack DNA sequence specificity and have non-selective activity, but these enzymes can be targeted to or excluded from selected genomic regions by numerous mechanisms

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including post-translational modifications and via interaction with instructive partner proteins [6]. For example, DNMT3A can form a complex with its catalytically inactive cofactor, DNA methyltransferase 3 like (DNMT3L), which stimulates DNMT3A activity during germline development [21,22], and in mouse embryonic stem cells (mESCs) [23]. At the N-terminus, DNMT3 enzymes contain a Pro-Trip-Trip-Pro (PWWP) domain, which can specifically recognize histone H3 molecules trimethylated at lysine 36, a histone modification enriched at actively transcribed gene bodies [24–26]. Another important domain for chromatin interaction is the ATRX-DNMT3-DNMT3L (ADD) domain, which enables DNMT3s to specifically recognize unmodified histone H3 lysine 4 [27–29], while methylation of histone H3 lysine 4 is known to inhibit this recognition [27,29,30]. Consequently, histone H3 lysine 4 tri-methylated (H3K4me3), a mark associated with active transcription, is thought to protect promoter CpGs from gaining a methyl group and thus prevents gene repression [28]. Once cell fate specification is completed, DNA methylation patterns are maintained after every mitotic cell division by the maintenance DNA methyltransferase 1 (DNMT1) [28]. Through interaction with proliferating cell nuclear antigen (PCNA), DNMT1 can localize to replication foci during the S-phase in dividing cells [31,32]. Interaction of DNMT1 with ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1) is necessary to guide DNMT1 to hemi-methylated DNA [33,34] (Figure 1).

For the correct placement of DNA methylation patterns and normal development, DNA methylation first needs to be erased during early preimplantation development and germline formation, a process often referred to as epigenetic reprogramming [35]. DNA methylation can be removed by two distinct mechanisms. Passive demethylation refers to the dilution of DNA methylation through cell divisions and can be caused by the absence or inhibition of DNMT1 [36,37], absence of UHRF1 [38] or delocalization of UHRF1 and DNMT1 to the cytoplasm [39,40]. Stella (Dppa3) is involved in the delocalization of UHRF1 in oocytes [41]. Active demethylation involves the removal of the methyl group from 5-methylcytosine (5mC) and is carried out by the ten-eleven translocation (TET) family of proteins. The three family members, TET1, TET2 and TET3, exhibit oxidizing activity and can catalyse the conversion of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [42,43]. In addition, thymine-DNA-glycosylase (TDG)-catalyzed base excision and the DNA base excision repair pathway can remove 5fC and 5caC so that unmodified cytosines can be incorporated [44] (Figure 1).

Immunodeficiency, centromeric instability, facial anomalies syndrome

Genetic defects in all three catalytically active DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B, have been associated with human congenital disorders [6,45,46], emphasizing the importance of DNA methylation for normal mammalian development. One of the earliest reports of abnormal DNA methylation patterns in disease was in patients with Immunodeficiency, Centromeric instability, Facial anomalies syndrome (ICF; OMIM 602900) approximately 40 years ago [47–49]. ICF syndrome is a rare, autosomal recessive disorder and less than 100 patients have been reported worldwide [50]. The main characteristics of the disease are unusual facial features, reduced levels or absence of serum immunoglobulins, and chromosome instability which is reflected in aberrant configurations of chromosomes 1, 9, and 16 in mitogen-stimulated lymphocytes [51]. The mild facial dysmorphisms frequently include hypertelorism, epicanthic folds, a flat nasal bridge, and low-set ears [52]. Most ICF patients are diagnosed at a young age, suffer from recurrent gastrointestinal and respiratory tract infections, sepsis and a failure to thrive, which often results in early childhood death [51]. An early study on ICF patient-derived peripheral blood found a lack of memory B and plasma cells that could explain the hypo- and agammaglobulinemia phenotype [53], and hematopoietic stem cell transplantation has been used to treat ICF patients [51,54]. DNA hypomethylation of pericentromeric satellite 2 and 3 repeats [55] is the molecular hallmark of the disease and makes ICF syndrome the first example of a human disorder linked to a constitutive defect in DNA methylation. The finding that the centromeric α-satellite repeats are heterogeneously affected by the loss of DNA methylation among ICF patients has led to the early recognition of genetic heterogeneity of the disease [55].

The genetics of ICF syndrome

In 1999, the first mutation underlying the ICF syndrome was described and found to be located in the gene that encodes DNMT3B [20,56,57]. As mentioned above, DNMT3B is a *de novo* methyltransferase involved in the establishment of DNA methylation patterns early in life and during cell differentiation. The majority of ICF1 patients carry missense mutations in the C-terminal part of DNMT3B, where the catalytic domain is located. In addition, *DNMT3B* nonsense and splice-site mutations have been reported, and all patients described to date appear to have

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impaired methyltransferase activity [58,59]. *DNMT3B* nonsense mutations only occur together with missense mutations, and it has been suggested that complete loss of DNMT3B catalytic activity is not compatible with life [60]. Consistent with this hypothesis, deletion of *Dnmt3b* in mice results in embryonic lethality [58], while mice carrying an ICF patient mutation in *Dnmt3b* are viable and resemble some of the phenotypic features of ICF syndrome patients [58,61]. Patients with *DNMT3B* mutations are designated ICF type 1 (ICF1) and account for approximately half of the known ICF patients. Three additional ICF syndrome-associated genes have been identified. Nonsense mutations in *zinc-finger and BTB domain-containing 24* (*ZBTB24*) are found in approximately 30% of ICF patients, referred to as ICF2 [62]. Twelve ICF cases with mutations in *cell division cycle associated 7* (*CDCA7*) or in *helicase, lymphoid specific* (*HELLS*) have been reported [63,64], and are referred to as ICF3 and ICF4, respectively. A few patients remain who showed molecular and phenotypic features characteristic of ICF syndrome but do not have mutations in the four known genes, and were referred to as ICFX [51].

Functions for ICF genes in DNA methylation pathways and beyond

The previously reported differences in repeat hypomethylation in ICF patients [55,65] can now be associated with the different genetic defects. The α-satellite repeat hypomethylation phenotype is shared between ICF2, ICF3, and ICF4 types [66], while DNA methylation levels of subtelomeric repeats are not affected in these patients [67]. In contrast, heavily methylated α-satellites [65] and the hypomethylation of subtelomeric repeats [67] are unique features of patients carrying *DNMT3B* mutations, and can be used to distinguish ICF1 from other ICF patients. Consistent with a function for DNMT3B in the establishment of DNA methylation in early development [11,68], ICF1 patients show promoter hypomethylation of germline genes [69] and loss of methylation at X-linked genes [66]; these sequences are not affected in ICF2, ICF3, and ICF4 patients [66]. Indeed, in both mice and humans, it has been shown that ZBTB24, CDCA7, and HELLS are involved in the DNMT1-dependent DNA methylation maintenance pathway. In mouse embryonic fibroblasts (MEFs), siRNA-mediated depletion of Zbtb24, Cdca7, and Hells resulted in loss of DNA methylation at minor satellite repeats [63]. Similarly, a progressive loss of DNA methylation at satellite repeats has been reported in human embryonic kidney (HEK) 293T cells knocked out for ZBTB24, CDCA7, or HELLS [70]. Interestingly, transient depletion of each of the four ICF factors in normal human primary fibroblasts did not result in hypomethylation of satellite 2 or subtelomeric sequences [67]. Biochemical studies have demonstrated that Hells can interact with Dnmt1 *in vitro* [71], and interactions between CDCA7 and HELLS [66,70,72], and CDCA7 and UHRF1 have also been reported [70]. A recent study in *Xenopus* showed that Cdca7 is required for localization of Hells to chromatin and that Cdca7 can stimulate Hells nucleosome remodeling activity [72]. Therefore, abnormal DNA methylation patterns in ICF3 patients could be the result of a defect in HELLS recruitment to chromatin [72]. Of note, some hypomethylated loci are shared between ICF patients from all four types [66], and there is evidence that Hells participates in Dnmt3a/b-mediated *de novo* methylation in MEFs and during the differentiation of mESCs [73,74]. Thus, roles for ZBTB24, CDCA7, and HELLS in *de novo* DNA methylation cannot be excluded, but are as yet undetermined.

Depending on the developmental stage, targeted genomic location, protein complex composition, cell cycle, or post-translational modifications, ZBTB24, CDCA7, and HELLS could also have functions that differ from DNA methylation regulation but are relevant to ICF syndrome pathology. For instance, both CDCA7 and HELLS have recently been linked to the classical non-homologous end joining (c-NHEJ) pathway [70]. CDCA7 was shown to interact with members of the c-NHEJ pathway, and depletion of both CDCA7 and HELLS in HEK293T cells was associated with c-NHEJ defects and delayed accumulation of Ku80 at sites of DNA damage [70]. Unresolved Holiday junctions have been suggested to underlie multiradial chromosome formation in ICF patients [75], and the findings by Unoki et al. [70] provide supporting evidence for this hypothesis.

In the case of ZBTB24, its function as a transcription factor and interaction with CDCA7 is now beginning to be understood. The functional connection between ZBTB24 and CDCA7, whereby ZBTB24 positively controls CDCA7 expression, was initially demonstrated in a mESC-based *Zbtb24* knockout model and in fibroblasts and T cells derived from ICF2 patients [76]. Subsequent studies have confirmed these results [66,70,77–79]. Recent genome-wide ChIP-seq studies reported that ZBTB24 is mainly enriched at CpG-rich promoters [77,78] and that its C2H2 zinc fingers direct sequence-specific binding [78]. Down-regulation of cdca7 has also been observed in a *zbtb24* knockout zebrafish model [80], demonstrating that *CDCA7* is probably a highly conserved ZBTB24 target.

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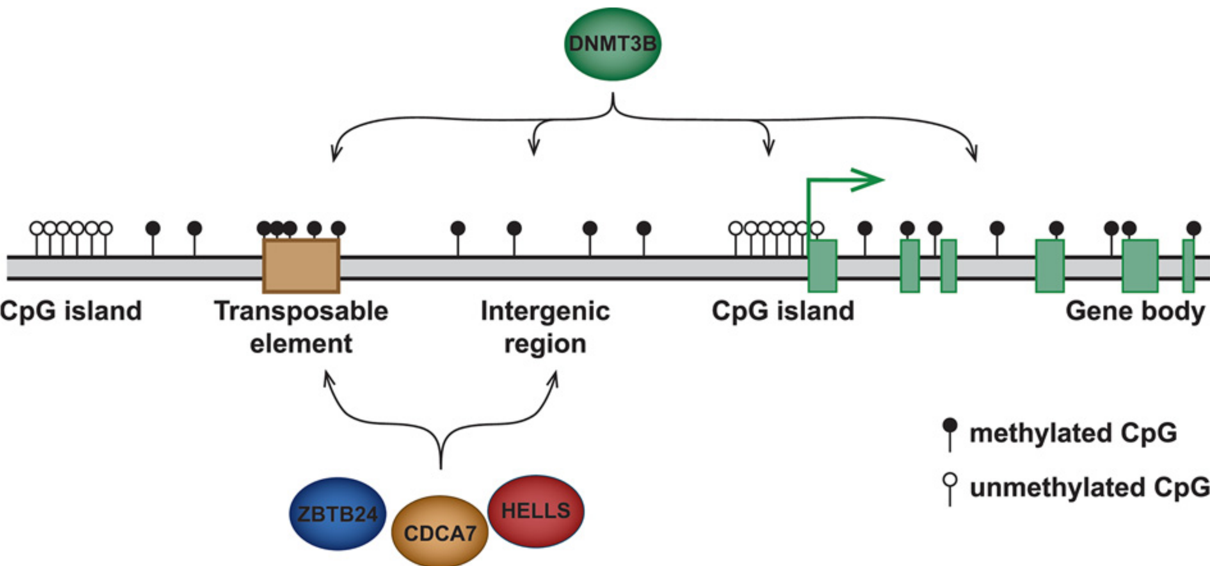

Figure 2. Schematic of the mammalian DNA methylation landscape and the putative influence of ICF genes on DNA methylation

The distribution of DNA methylation across different genomic elements, such as transposable elements, intergenic regions, CGI and gene bodies. DNMT3B can influence DNA methylation establishment genome-wide, with the exception of CGI that are usually protected from DNA methylation. ZBTB24, CDCA7, and HELLS contribute to DNA methylation maintenance and possibly establishment mainly at intergenic regions and repetitive elements. The empty circles represent unmethylated cytosines, while filled circles represent methylated cytosines (adapted from: https://commons.wikimedia.org/wiki/File:DNAme_landscape.png).

ICF syndrome patients have an altered DNA methylation landscape

Pericentromeric satellites 2, 3 and centromeric α-satellites are classical targets of DNA hypomethylation in ICF syndrome patients. In addition, abnormal DNA methylation of subtelomeric repeats is a characteristic feature of ICF1 patients [67,81,82]. Initially ICF-related DNA hypomethylation appeared to be confined to the repetitive compartment of the genome [65,67,83–87], and only a few single copy loci exhibiting DNA hypomethylation in ICF patients were known [69,88–92]. Rapid developments in next-generation sequencing technologies and the identification of *ZBTB24*, *CDCA7*, and *HELLS* as ICF disease genes have stimulated research in this area, allowing a more detailed view of the ICF-related methylome. The first genome-wide DNA methylation map, generated from ICF1 patient-derived cultured lymphoblastoid cell lines (LCLs), has revealed widespread hypomethylation and a 41% reduction in global methylation levels when compared with a control individual [93]. All autosomes were equally affected and loss of methylation was not restricted to repetitive regions but detected along all genomic features including promoters, gene bodies, and intergenic regions [93] (Figure 2). A later study, using independent ICF1 patient-derived cultured LCLs and reduced-representation bisulfite sequencing (RRBS), reported similar findings with respect to global DNA methylation alterations [94]. In mESCs, it has been reported that Dnmt3b is the main enzyme responsible for genic DNA methylation [24], and mESCs lacking Dnmt3b lost DNA methylation across exons and introns [14]. Consistent with this, ICF1 patients show hypomethylation of gene bodies [94]. It has also been shown that most DNMT3B binding occurs at intragenic positions of genes and that DNMT3B binding is not impaired upon the introduction of an ICF1 mutation in the catalytic domain [94]. Intriguingly, DNMT3B patients with a homozygous missense mutation close to the PWWP domain have been reported [95], demonstrating that the ICF syndrome phenotype can be recapitulated by mutations in the regulatory N-terminal part of DNMT3B. Patients carrying the PWWP mutation show DNA hypomethylation of satellite 2 repeats [95], and it will be interesting to determine whether they have additional methylation defects that are similar or different from the ones that have been described for ICF1 patients with impaired DNMT3B catalytic activity. Of note, introduction of the PWWP mutation into mESCs resulted in loss of Dnmt3b recruitment to histone H3 lysine 36 tri-methylated (H3K36me3)-decorated gene bodies [24].

While ICF1 patients show prominent loss of DNA methylation in CpG-rich regions (CGI and CpG shores) and gene bodies, it has become evident that in addition to the satellite repeats, CpG-poor, heterochromatic regions that are

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often referred to as 'open sea', are predominantly affected in ICF2, 3, and 4 patients (Figure 2). This was only recently revealed in a comprehensive study comparing 'primary' whole blood methylomes generated from 15 patients comprising samples from all four ICF types and ten gender- and age-matched controls [66]. Many of the hypomethylated CpGs in ICF2, 3, and 4 patients mapped to late-replicating regions and were found to be located in the open sea regions of the genome [66], which are characterized by low CpG density [96]. Heterochromatin is usually characterized by the presence of the repressive histone modifications histone H3 lysine 9 di/tri-methylated (H3K9me2 and H3K9me3), and binding of HP1 [97]. DNA methylation can contribute to heterochromatin formation through interaction with components of the chromatin remodeling and histone modification machinery, since DNMTs cannot bind and efficiently methylate nucleosomal DNA [98,99]. For instance, DNMTs can interact with the chromatin remodeler HELLS (also known as LSH) [71] or histone-modifying enzymes, such as suppressor of variegation 3-9 homolog 1 (SUV39H1) [100], and the H3K9me3 reader, UHRF1 [101]. In addition, DNMT3B can be directly recruited to centromeric heterochromatin by centromere protein C (CENPC) [102] or the linker histone H1 [103,104]. How ZBTB24, CDCA7, and HELLS contribute to DNA methylation and possibly heterochromatin formation at (peri)centromeric repeats and open sea regions, and why these sites are specifically affected in ICF2, ICF3 and ICF4 patients, remains an open question. Gene regulatory elements are also located in open sea regions, and it has recently been suggested that short-term depletion of Hells in MEFs is associated with increased chromatin accessibility at selected genes and enhancers [105]. Hypomethylation of large chromosomal domains has been observed in MEFs derived from *Hells*$^{-/-}$ embryos, and a function for Hells in controlling DNA methylation in a nuclear compartment partly defined by lamina-associated domains (LADs) has been proposed [106]. Given the functional connections between the three disease genes and the overlapping hypomethylation phenotype in ICF2–4 type patients it will be interesting to test whether ZBTB24, CDCA7, and HELLS may also have roles in LAD and/or enhancer regulation.

Members of big gene cluster families including the olfactory receptor genes and the clustered protocadherins (*PCDH*) exhibit aberrant DNA methylation in all four ICF patient types [66]. Epigenetic mechanisms play important roles in the regulation of gene clusters [107] and their dysregulation could be associated with ICF syndrome phenotypes. For example, the clustered *PCDH* genes, which are predominantly expressed in the nervous system, are key components of neuronal diversity [108]. In mice, it has been shown that Dnmt3b is essential for the *de novo* methylation of *Pcdh* cluster genes in early embryonic development [109]. Defects in *Pcdh* cluster regulation have also been reported upon neuronal ablation of the H3K9me3 histone methyltransferase, Setdb1, in mice [110], suggesting cross-talk between DNA methylation and histone modification pathways. In addition, Setdb1 is important for preserving a large topologically associated domain (TAD) encompassing the *Pcdh* locus [110]. Since intellectual disability is a common feature in ICF syndrome patients [51], it will be interesting to determine whether defects in *PCDH* gene cluster regulation can contribute to aspects of the disease.

Finally, it is worth mentioning that abnormal DNA hypermethylation of different genomic features including promoters has been observed in ICF patients [66,93,94]. Although its relevance is not yet understood, given the well-established function of DNA methylation in gene silencing, it can be envisioned that DNA hypermethylation may also contribute to the phenotypes observed in ICF syndrome patients.

Linking aberrant DNA methylation to ICF syndrome phenotype

Indeed, the challenge now is to link DNMT3B-, ZBTB24-, CDCA7-, and HELLS-related genome-wide DNA methylation alterations to gene expression changes that could explain ICF patient phenotypes. For a large part, progress in this area has been hampered by the scarcity of ICF patient-derived tissues or cell lines. Consequently, an integrated analysis of genome-wide DNA methylation and gene expression data has so far only been reported for cultured LCLs from ICF1 patients [94]. That study showed that aberrant gene body methylation in ICF1 patients was associated with alternative transcription start site (TSS) usage, the regulation of sense-antisense transcription and alternative exon splicing [94]. A similar function for gene body methylation in preventing aberrant transcript initiation has been shown in mESCs and could be ascribed to the catalytic activity of Dnmt3b [14]. While it is challenging to directly translate observations from model systems or cultured cells to the ICF patient situation, it is feasible that spurious transcript initiation could critically affect phenotype, when associated with the production of aberrant proteins.

In addition to the ICF1-specific anomalous gene body methylation, ICF patient methylomes are characterized by hypomethylation of the heterochromatic compartment of the genome, as discussed above. By definition, such regions are transcriptionally inert and enriched for repressive epigenetic marks [111]. Genome-wide expression profiling in ICF1 patient-derived LCLs identified modest changes in gene expression outside gene bodies, although global DNA hypomethylation was observed [94]. Indeed, histone modification ChIP-seq revealed increased histone H3 lysine

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27 three-methylated (H3K27me3) levels predominantly at hypomethylated CpGs and a compensatory function for this repressive mark has been suggested [94]. Re-distribution of H3K27me3 at gene cluster regions and surprisingly mild changes in gene expression have also been reported in *Hells*$^{-/-}$ MEFs, consistent with a redundancy in epigenetic silencing pathways upon loss of DNA methylation [106]. Whether these findings reflect convergent functions of Dnmt3b and Hells in *de novo* DNA methylation, or are a unifying feature in all ICF patients, remains to be determined.

Conclusion

Described as a disorder of 'variable immunodeficiency, facial anomalies and centromeric heterochromatin instability' approximately 40 years ago, the ICF syndrome has fascinated scientists ever since. Aberrant DNA methylation is probably involved in the causation of the disease, but what types of changes in DNA methylation patterns lead to the phenotypic aspects of the disorder remains an open question. A great deal has been learned about the genetic defects underlying ICF syndrome and the genome-wide epigenetic consequences thereof. In addition, the molecular interactions between the four disease genes and their functions in cellular processes are beginning to be understood. Gaining further insights into these mechanisms through increasingly sophisticated genetics, biochemistry and genomics approaches will enable us to progress toward a fuller understanding of how the dysfunction of four different proteins can lead to the same disease phenotype.

Summary

- ICF is a rare primary immunodeficiency characterized by multiradiated chromosomes 1, 9 and 16, and hypomethylation of pericentromeric satellite repeats 2 and 3, and has been linked to defects in four genes, namely, *DNMT3B*, *ZBTB24*, *CDCA7*, and *HELLS*.
- Genomic regions that are commonly affected in all four ICF types include members of big gene cluster families such as the clustered *PCDH*.
- Anomalous gene body methylation is specific to ICF1 patients and has been associated with aberrant transcript initiation.
- In addition to α-satellite repeat sequences, CpG-poor regions enriched for heterochromatin marks are predominantly affected by DNA hypomethylation in ICF2, 3, and 4 patients.

Author Contribution

M.V. and L.D. both wrote the review.

Funding

This work was supported by the Leiden University Medical Centre (LUMC Fellowship) (to L.D.); and the Dutch Research Council [grant number NWO-ZonMw-Vidi 91718350 (to L.D.)].

Competing Interests

The authors declare that there are no competing interests associated with this manuscript.

Abbreviations

ADD, ATRX-DNMT3-DNMT3L; CDCA7, cell division cycle associated 7; CGI, CpG island; CENPC, centromeric heterochromatin by centromere protein C; c-NHEJ, classical non-homologous end joining; DNMT1, DNA methyltransferase 1; DNMT3A, DNA methyltransferase 3 alpha; DNMT3B, DNA methyltransferase 3 beta; DNMT3L, DNA methyltransferase 3 like; HELLS/LSH , helicase lymphoid specific; HEK, human embryonic kidney; HP1, heterochromatin protein 1; H3K27me3, histone H3 lysine 27 tri-methylated; H3K36me3, histone H3 lysine 36 tri-methylated; H3K4me3, histone H3 lysine 4 tri-methylated; H3K9me2/3, histone H3 lysine 9 di/tri-methylated; ICF, Immunodeficiency, Centromeric instability, Facial anomalies syndrome; LAD, lamina-associated domain; LCL, lymphoblastoid cell line; MEF, mouse embryonic fibroblast; mESC, mouse embryonic stem cell; PCHD, protocadherin; PCNA, proliferating cell nuclear antigen; PWWP, Pro-Trip-Trip-Pro domain; RRBS, reduced-representation bisulfite sequencing; Setdb1, SET domain bifurcated histone lysine methyltransferase 1; Stella/Dppa3,

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developmental pluripotency-associated 3; SUV39H1, suppressor of variegation 3-9 homolog 1; TAD, topologically associated domain; TDG, thymine-DNA-glycosylase; TET, ten-eleven translocation; TSS, transcription start site; UHRF1, ubiquitin-like, containing PHD and RING finger domains 1; ZBTB24, zinc-finger and BTB domain-containing 24; 5caC, 5-carboxylcytosine; 5fC, 5-formylcyosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine.

References

1 Hotchkiss, R.D. (1948) The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. *J. Biol. Chem.* **175**, 315–332
2 Holliday, R. and Pugh, J.E. (1975) DNA modification mechanisms and gene activity during development. *Science* **187**, 226–232, https://doi.org/10.1126/science.1111098
3 Razin, A. and Riggs, A.D. (1980) DNA methylation and gene function. *Science* **210**, 604–610, https://doi.org/10.1126/science.6254144
4 Greenberg, M.V.C. and Bourc'his, D. (2019) The diverse roles of DNA methylation in mammalian development and disease. *Nat. Rev. Mol. Cell Biol.* **10**, 590–607, https://doi.org/10.1038/s41580-019-0159-6
5 Schübeler, D. (2015) Function and information content of DNA methylation. *Nature* **517**, 321, https://doi.org/10.1038/nature14192
6 Lyko, F. (2018) The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. *Nat. Rev. Genet.* **19**, 81–92, https://doi.org/10.1038/nrg.2017.80
7 Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P. et al. (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562, https://doi.org/10.1038/nature01262
8 Deniz, Ö, Frost, J.M. and Branco, M.R. (2019) Regulation of transposable elements by DNA modifications. *Nat. Rev. Genet.* **20**, 417–431, https://doi.org/10.1038/s41576-019-0106-6
9 Hancks, D.C. and Kazazian, Jr, H.H. (2016) Roles for retrotransposon insertions in human disease. *Mobile DNA* **7**, 9, https://doi.org/10.1186/s13100-016-0065-9
10 Edwards, J.R., Yarychkivska, O., Boulard, M. and Bestor, T.H. (2017) DNA methylation and DNA methyltransferases. *Epigenetics Chromatin* **10**, 23
11 Borgel, J., Guibert, S., Li, Y., Chiba, H., Schubeler, D., Sasaki, H. et al. (2010) Targets and dynamics of promoter DNA methylation during early mouse development. *Nat. Genet.* **42**, 1093–1100, https://doi.org/10.1038/ng.708
12 Liao, J., Karnik, R., Gu, H., Ziller, M.J., Clement, K., Tsankov, A.M. et al. (2015) Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. *Nat. Genet.* **47**, 469–478, https://doi.org/10.1038/ng.3258
13 Straussman, R., Nejman, D., Roberts, D., Steinfeld, I., Blum, B., Benvenisty, N. et al. (2009) Developmental programming of CpG island methylation profiles in the human genome. *Nat. Struct. Mol. Biol.* **16**, 564–571
14 Neri, F., Rapelli, S., Krepelova, A., Incarnato, D., Parlato, C., Basile, G. et al. (2017) Intragenic DNA methylation prevents spurious transcription initiation. *Nature* **543**, 72–77, https://doi.org/10.1038/nature21373
15 Maunakea, A.K., Chepelev, I., Cui, K. and Zhao, K. (2013) Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res.* **23**, 1256–1269, https://doi.org/10.1038/cr.2013.110
16 Yearim, A., Gelfman, S., Shayevitch, R., Melcer, S., Glaich, O., Mallm, J.P. et al. (2015) HP1 is involved in regulating the global impact of DNA methylation on alternative splicing. *Cell Rep.* **10**, 1122–1134, https://doi.org/10.1016/j.celrep.2015.01.038
17 Shukla, S., Kavak, E., Gregory, M., Imashimizu, M., Shutinoski, B., Kashlev, M. et al. (2011) CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature* **479**, 74–79, https://doi.org/10.1038/nature10442
18 Jones, P.A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* **13**, 484–492, https://doi.org/10.1038/nrg3230
19 Auclair, G., Guibert, S., Bender, A. and Weber, M. (2014) Ontogeny of CpG island methylation and specificity of DNMT3 methyltransferases during embryonic development in the mouse. *Genome Biol.* **15**, 545, https://doi.org/10.1186/s13059-014-0545-5
20 Okano, M., Bell, D.W., Haber, D.A. and Li, E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257, https://doi.org/10.1016/S0092-8674(00)81656-6
21 Chédin, F., Lieber, M.R. and Hsieh, C-L (2002) The DNA methyltransferase-like protein DNMT3L stimulates *de novo* methylation by Dnmt3a. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 16916–16921
22 Bourc'his, D. and Bestor, T.H. (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* **431**, 96–99, https://doi.org/10.1038/nature02886
23 Veland, N., Lu, Y., Hardikar, S., Gaddis, S., Zeng, Y., Liu, B. et al. (2018) DNMT3L facilitates DNA methylation partly by maintaining DNMT3A stability in mouse embryonic stem cells. *Nucleic Acids Res.* **47**, 152–167, https://doi.org/10.1093/nar/gky947
24 Baubec, T., Colombo, D.F., Wirbelauer, C., Schmidt, J., Burger, L., Krebs, A.R. et al. (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**, 243–247, https://doi.org/10.1038/nature14176
25 Morselli, M., Pastor, W.A., Montanini, B., Nee, K., Ferrari, R., Fu, K. et al. (2015) In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. *eLife* **4**, e06205, https://doi.org/10.7554/eLife.06205
26 Dhayalan, A., Rajavelu, A., Rathert, P., Tamas, R., Jurkowska, R.Z., Ragozin, S. et al. (2010) The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J. Biol. Chem.* **285**, 26114–26120, https://doi.org/10.1074/jbc.M109.089433
27 Ooi, S.K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z. et al. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**, 714–717, https://doi.org/10.1038/nature05987
28 Du, J., Johnson, L.M., Jacobsen, S.E. and Patel, D.J. (2015) DNA methylation pathways and their crosstalk with histone methylation. *Nat. Rev. Mol. Cell Biol.* **16**, 519–532, https://doi.org/10.1038/nrm4043

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https://doi.org/10.1042/EBC20190035

29 Guo, X., Wang, L., Li, J., Ding, Z., Xiao, J., Yin, X. et al. (2015) Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* **517**, 640–644, https://doi.org/10.1038/nature13899
30 Otani, J., Nankumo, T., Arita, K., Inamoto, S., Ariyoshi, M. and Shirakawa, M. (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep.* **10**, 1235–1241, https://doi.org/10.1038/embor.2009.218
31 Chuang, L.S., Ian, H.I., Koh, T.W., Ng, H.H., Xu, G. and Li, B.F. (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* **277**, 1996–2000, https://doi.org/10.1126/science.277.5334.1996
32 Leonhardt, H., Page, A.W., Weier, H.U. and Bestor, T.H. (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell* **71**, 865–873, https://doi.org/10.1016/0092-8674(92)90561-P
33 Bostick, M., Kim, J.K., Esteve, P.O., Clark, A., Pradhan, S. and Jacobsen, S.E. (2007) UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317**, 1760–1764, https://doi.org/10.1126/science.1147939
34 Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A. et al. (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908–912, https://doi.org/10.1038/nature06397
35 Hackett, J.A. and Surani, M.A. (2013) DNA methylation dynamics during the mammalian life cycle. *Phil. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **368**, 20110328, https://doi.org/10.1098/rstb.2011.0328
36 Seisenberger, S., Peat, J.R., Hore, T.A., Santos, F., Dean, W. and Reik, W. (2013) Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Phil. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **368**, 20110330, https://doi.org/10.1098/rstb.2011.0330
37 Li, E. and Zhang, Y. (2014) DNA methylation in mammals. *Cold Spring Harb. Perspect. Biol.* **6**, a019133, https://doi.org/10.1101/cshperspect.a019133
38 Kagiwada, S., Kurimoto, K., Hirota, T., Yamaji, M. and Saitou, M. (2013) Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. *EMBO J.* **32**, 340–353, https://doi.org/10.1038/emboj.2012.331
39 Maenohara, S., Unoki, M., Toh, H., Ohishi, H., Sharif, J., Koseki, H. et al. (2017) Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. *PLoS Genet.* **13**, e1007042, https://doi.org/10.1371/journal.pgen.1007042
40 Hirasawa, R., Chiba, H., Kaneda, M., Tajima, S., Li, E., Jaenisch, R. et al. (2008) Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev.* **22**, 1607–1616
41 Li, Y., Zhang, Z., Chen, J., Liu, W., Lai, W., Liu, B. et al. (2018) Stella safeguards the oocyte methylome by preventing *de novo* methylation mediated by DNMT1. *Nature* **564**, 136–140, https://doi.org/10.1038/s41586-018-0751-5
42 Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y. et al. (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930–935, https://doi.org/10.1126/science.1170116
43 Kriaucionis, S. and Tahiliani, M. (2014) Expanding the epigenetic landscape: novel modifications of cytosine in genomic DNA. *Cold Spring Harb. Perspect. Biol.* **6**, a018630, https://doi.org/10.1101/cshperspect.a018630
44 Wu, X. and Zhang, Y. (2017) TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat. Rev. Genet.* **18**, 517–534, https://doi.org/10.1038/nrg.2017.33
45 Velasco, G. and Francastel, C. (2019) Genetics meets DNA methylation in rare diseases. *Clin. Genet.* **95**, 210–220, https://doi.org/10.1111/cge.13480
46 Heyn, P., Logan, C.V., Fluteau, A., Challis, R.C., Auchynnikava, T., Martin, C.A. et al. (2019) Gain-of-function DNMT3A mutations cause microcephalic dwarfism and hypermethylation of Polycomb-regulated regions. *Nat. Genet.* **51**, 96–105, https://doi.org/10.1038/s41588-018-0274-x
47 Maraschio, P., Zuffardi, O., Dalla Fior, T. and Tiepolo, L. (1988) Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial anomalies: the ICF syndrome. *J. Med. Genet.* **25**, 173–180, https://doi.org/10.1136/jmg.25.3.173
48 Hulten, M. (1978) Selective somatic pairing and fragility at 1q12 in a boy with common variable immuno deficiency. *Clin. Genet.* **14**, 294, https://doi.org/10.1111/j.1399-0004.1978.tb02170.x
49 Tiepolo, L., Maraschio, P., Gimelli, G., Cuoco, C., Gargani, G.F. and Romano, C. (1979) Multibranched chromosomes 1, 9, and 16 in a patient with combined IgA and IgE deficiency. *Hum. Genet.* **51**, 127–137, https://doi.org/10.1007/BF00287166
50 van den Boogaard, M.L., Thijssen, P.E., Aytekin, C., Licciardi, F., Kiykim, A.A., Spossito, L. et al. (2017) Expanding the mutation spectrum in ICF syndrome: evidence for a gender bias in ICF2. *Clin. Genet.* **92**, 380–387, https://doi.org/10.1111/cge.12979
51 Weemaes, C.M., van Tol, M.J., Wang, J., van Ostaijen-ten Dam, M.M., van Eggermond, M.C., Thijssen, P.E. et al. (2013) Heterogeneous clinical presentation in ICF syndrome: correlation with underlying gene defects. *Eur. J. Hum. Genet.* **21**, 1219–1225, https://doi.org/10.1038/ejhg.2013.40
52 Ehrlich, M., Sanchez, C., Shao, C., Nishiyama, R., Kehrl, J., Kuick, R. et al. (2008) ICF, an immunodeficiency syndrome: DNA methyltransferase 3B involvement, chromosome anomalies, and gene dysregulation. *Autoimmunity* **41**, 253–271, https://doi.org/10.1080/08916930802024202
53 Blanco-Betancourt, C.E., Moncla, A., Milili, M., Jiang, Y.L., Viegas-Pequignot, E.M., Roquelaure, B. et al. (2004) Defective B-cell-negative selection and terminal differentiation in the ICF syndrome. *Blood* **103**, 2683–2690, https://doi.org/10.1182/blood-2003-08-2632
54 Gennery, A.R., Slatter, M.A., Bredius, R.G., Hagleitner, M.M., Weemaes, C., Cant, A.J. et al. (2007) Hematopoietic stem cell transplantation corrects the immunologic abnormalities associated with immunodeficiency-centromeric instability-facial dysmorphism syndrome. *Pediatrics* **120**, e1341–4, https://doi.org/10.1542/peds.2007-0640
55 Jeanpierre, M., Turleau, C., Aurias, A., Prieur, M., Ledeist, F., Fischer, A. et al. (1993) An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. *Hum. Mol. Genet.* **2**, 731–735, https://doi.org/10.1093/hmg/2.6.731
56 Xu, G.L., Bestor, T.H., Bourc'his, D., Hsieh, C.L., Tommerup, N., Bugge, M. et al. (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402**, 187–191, https://doi.org/10.1038/46052
57 Hansen, R.S., Wijmenga, C., Luo, P., Stanek, A.M., Canfield, T.K., Weemaes, C.M. et al. (1999) The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14412–14417, https://doi.org/10.1073/pnas.96.25.14412
58 Ueda, Y., Okano, M., Williams, C., Chen, T., Georgopoulos, K. and Li, E. (2006) Roles for Dnmt3b in mammalian development: a mouse model for the ICF syndrome. *Development* **133**, 1183–1192, https://doi.org/10.1242/dev.02293

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59 Gagliardi, M., Strazzullo, M. and Matarazzo, M.R. (2018) DNMT3B functions: novel insights from human disease. *Front. Cell Dev. Biol.* **6**, 140, https://doi.org/10.3389/fcell.2018.00140
60 Walton, E.L., Francastel, C. and Velasco, G. (2011) Maintenance of DNA methylation: Dnmt3b joins the dance. *Epigenetics* **6**, 1373–1377, https://doi.org/10.4161/epi.6.11.17978
61 Velasco, G., Hube, F., Rollin, J., Neuillet, D., Philippe, C., Bouzinba-Segard, H. et al. (2010) Dnmt3b recruitment through E2F6 transcriptional repressor mediates germ-line gene silencing in murine somatic tissues. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 9281–9286, https://doi.org/10.1073/pnas.1000473107
62 de Greef, J.C., Wang, J., Balog, J., den Dunnen, J.T., Frants, R.R., Straasheijm, K.R. et al. (2011) Mutations in ZBTB24 are associated with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. *Am. J. Hum. Genet.* **88**, 796–804, https://doi.org/10.1016/j.ajhg.2011.04.018
63 Thijssen, P.E., Ito, Y., Grillo, G., Wang, J., Velasco, G., Nitta, H. et al. (2015) Mutations in CDCA7 and HELLS cause immunodeficiency-centromeric instability-facial anomalies syndrome. *Nat. Commun.* **6**, 7870, https://doi.org/10.1038/ncomms8870
64 Alghamdi, H.A., Tashkandi, S.A., Alidrissi, E.M., Aledielah, R.D., AlSaidi, K.A., Alharbi, E.S. et al. (2018) Three types of immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome identified by whole-exome sequencing in Saudi hypogammaglobulinemia patients: clinical, molecular, and cytogenetic features. *J. Clin. Immunol.* **38**, 847–853, https://doi.org/10.1007/s10875-018-0569-9
65 Jiang, Y.L., Rigolet, M., Bourc'his, D., Nigon, F., Bokesoy, I., Fryns, J.P. et al. (2005) DNMT3B mutations and DNA methylation defect define two types of ICF syndrome. *Hum. Mutat.* **25**, 56–63, https://doi.org/10.1002/humu.20113
66 Velasco, G., Grillo, G., Touleimat, N., Ferry, L., Ivkovic, I., Ribierre, F. et al. (2018) Comparative methylome analysis of ICF patients identifies heterochromatin loci that require ZBTB24, CDCA7 and HELLS for their methylated state. *Hum. Mol. Genet.* **27**, 2409–2424, https://doi.org/10.1093/hmg/ddy130
67 Toubiana, S., Velasco, G., Chityat, A., Kaindl, A.M., Hershtig, N., Tzur-Gilat, A. et al. (2018) Subtelomeric methylation distinguishes between subtypes of Immunodeficiency, centromeric instability and Facial anomalies syndrome. *Hum. Mol. Genet.* **27**, 3568–3581, https://doi.org/10.1093/hmg/ddy265
68 Oda, M., Yamagiwa, A., Yamamoto, S., Nakayama, T., Tsumura, A., Sasaki, H. et al. (2006) DNA methylation regulates long-range gene silencing of an X-linked homeobox gene cluster in a lineage-specific manner. *Genes Dev.* **20**, 3382–3394
69 Velasco, G., Walton, E.L., Sterlin, D., Hedouin, S., Nitta, H., Ito, Y. et al. (2014) Germline genes hypomethylation and expression define a molecular signature in peripheral blood of ICF patients: implications for diagnosis and etiology. *Orphanet. J. Rare Dis.* **9**, 56, https://doi.org/10.1186/1750-1172-9-56
70 Unoki, M., Funabiki, H., Velasco, G., Francastel, C. and Sasaki, H. (2019) CDCA7 and HELLS mutations undermine nonhomologous end joining in centromeric instability syndrome. *J. Clin. Invest.* **129**, 78–92, https://doi.org/10.1172/JCI99751
71 Dunican, D.S., Pennings, S. and Meehan, R.R. (2015) Lsh is essential for maintaining global DNA methylation levels in amphibia and fish and interacts directly with Dnmt1. *BioMed Res. Int.* **2015**, 740637, https://doi.org/10.1155/2015/740637
72 Jenness, C., Giunta, S., Muller, M.M., Kimura, H., Muir, T.W. and Funabiki, H. (2018) HELLS and CDCA7 comprise a bipartite nucleosome remodeling complex defective in ICF syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E876–E885, https://doi.org/10.1073/pnas.1717509115
73 Zhu, H., Geiman, T.M., Xi, S., Jiang, Q., Schmidtmann, A., Chen, T. et al. (2006) Lsh is involved in de novo methylation of DNA. *EMBO J.* **25**, 335–345, https://doi.org/10.1038/sj.emboj.7600925
74 Myant, K., Termanis, A., Sundaram, A.Y., Boe, T., Li, C., Merusi, C. et al. (2011) LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. *Genome Res.* **21**, 83–94, https://doi.org/10.1101/gr.108498.110
75 Tuck-Muller, C.M., Narayan, A., Tsien, F., Smeets, D.F., Sawyer, J., Fiala, E.S. et al. (2000) DNA hypomethylation and unusual chromosome instability in cell lines from ICF syndrome patients. *Cytogenet. Cell Genet.* **89**, 121–128, https://doi.org/10.1159/000015590
76 Wu, H., Thijssen, P.E., de Klerk, E., Vonk, K.K., Wang, J., den Hamer, B. et al. (2016) Converging disease genes in ICF syndrome: ZBTB24 controls expression of CDCA7 in mammals. *Hum. Mol. Genet.* **25**, 4041–4051, https://doi.org/10.1093/hmg/ddw243
77 Thompson, J.J., Kaur, R., Sosa, C.P., Lee, J.H., Kashiwagi, K., Zhou, D. et al. (2018) ZBTB24 is a transcriptional regulator that coordinates with DNMT3B to control DNA methylation. *Nucleic Acids Res.* **46**, 10034–10051, https://doi.org/10.1093/nar/gky682
78 Ren, R., Hardikar, S., Horton, J.R., Lu, Y., Zeng, Y., Singh, A.K. et al. (2019) Structural basis of specific DNA binding by the transcription factor ZBTB24. *Nucleic Acids Res.* **16**, 8388–8398, https://doi.org/10.1093/nar/gkz557
79 Wu, H., Vonk, K.K.D., van der Maarel, S.M., Santen, G.W.E. and Daxinger, L. (2019) A functional assay to classify ZBTB24 missense variants of unknown significance. *Hum. Mutat.* **40**, 1077–1083
80 Rajshekar, S., Yao, J., Arnold, P.K., Payne, S.G., Zhang, Y., Bowman, T.V. et al. (2018) Pericentromeric hypomethylation elicits an interferon response in an animal model of ICF syndrome. *eLife* **7**, https://doi.org/10.7554/eLife.39658
81 Simo-Riudalbas, L., Diaz-Lagares, A., Gatto, S., Gagliardi, M., Crujeiras, A.B., Matarazzo, M.R. et al. (2015) Genome-wide DNA methylation analysis identifies novel hypomethylated non-pericentromeric genes with potential clinical implications in ICF syndrome. *PLoS ONE* **10**, e0132517, https://doi.org/10.1371/journal.pone.0132517
82 Sagie, S., Ellran, E., Katzir, H., Shaked, R., Yehezkel, S., Laevsky, I. et al. (2014) Induced pluripotent stem cells as a model for telomeric abnormalities in ICF type I syndrome. *Hum. Mol. Genet.* **23**, 3629–3640, https://doi.org/10.1093/hmg/ddu071
83 Miniou, P., Jeanpierre, M., Bourc'his, D., Coutinho Barbosa, A.C., Blanquet, V. and Viegas-Pequignot, E. (1997) alpha-satellite DNA methylation in normal individuals and in ICF patients: heterogeneous methylation of constitutive heterochromatin in adult and fetal tissues. *Hum. Genet.* **99**, 738–745, https://doi.org/10.1007/s004390050441
84 Yehezkel, S., Segev, Y., Viegas-Pequignot, E., Skorecki, K. and Selig, S. (2008) Hypomethylation of subtelomeric regions in ICF syndrome is associated with abnormally short telomeres and enhanced transcription from telomeric regions. *Hum. Mol. Genet.* **17**, 2776–2789, https://doi.org/10.1093/hmg/ddn177

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85 Deng, Z., Campbell, A.E. and Lieberman, P.M. (2010) TERRA, CpG methylation and telomere heterochromatin: lessons from ICF syndrome cells. *Cell Cycle* **9**, 69–74, https://doi.org/10.4161/cc.9.1.10358

86 Miniou, P., Bourc'his, D., Molina Gomes, D., Jeanpierre, M. and Viegas-Pequignot, E. (1997) Undermethylation of Alu sequences in ICF syndrome: molecular and *in situ* analysis. *Cytogenet. Cell Genet.* **77**, 308–313, https://doi.org/10.1159/000134605

87 Kondo, T., Bobek, M.P., Kuick, R., Lamb, B., Zhu, X., Narayan, A. et al. (2000) Whole-genome methylation scan in ICF syndrome: hypomethylation of non-satellite DNA repeats D4Z4 and NBL2. *Hum. Mol. Genet.* **9**, 597–604, https://doi.org/10.1093/hmg/9.4.597

88 Miniou, P., Jeanpierre, M., Blanquet, V., Sibella, V., Bonneau, D., Herbelin, C. et al. (1994) Abnormal methylation pattern in constitutive and facultative (X inactive chromosome) heterochromatin of ICF patients. *Hum. Mol. Genet.* **3**, 2093–2102, https://doi.org/10.1093/hmg/3.12.2093

89 Bourc'his, D., Miniou, P., Jeanpierre, M., Molina Gomes, D., Dupont, J., De Saint-Basile, G. et al. (1999) Abnormal methylation does not prevent X inactivation in ICF patients. *Cytogenet. Cell Genet.* **84**, 245–252, https://doi.org/10.1159/000015269

90 Schuffenhauer, S., Bartsch, O., Stumm, M., Buchholz, T., Petropoulou, T., Kraft, S. et al. (1995) DNA, FISH and complementation studies in ICF syndrome: DNA hypomethylation of repetitive and single copy loci and evidence for a trans acting factor. *Hum. Genet.* **96**, 562–571, https://doi.org/10.1007/BF00197412

91 Tao, Q., Huang, H., Geiman, T.M., Lim, C.Y., Fu, L., Qiu, G.H. et al. (2002) Defective *de novo* methylation of viral and cellular DNA sequences in ICF syndrome cells. *Hum. Mol. Genet.* **11**, 2091–2102, https://doi.org/10.1093/hmg/11.18.2091

92 Jin, B., Tao, Q., Peng, J., Soo, H.M., Wu, W., Ying, J. et al. (2008) DNA methyltransferase 3B (DNMT3B) mutations in ICF syndrome lead to altered epigenetic modifications and aberrant expression of genes regulating development, neurogenesis and immune function. *Hum. Mol. Genet.* **17**, 690–709, https://doi.org/10.1093/hmg/ddm341

93 Heyn, H., Vidal, E., Sayols, S., Sanchez-Mut, J.V., Moran, S., Medina, I. et al. (2012) Whole-genome bisulfite DNA sequencing of a DNMT3B mutant patient. *Epigenetics* **7**, 542–550, https://doi.org/10.4161/epi.20523

94 Gatto, S., Gagliardi, M., Franzese, M., Leppert, S., Papa, M., Cammisa, M. et al. (2017) ICF-specific DNMT3B dysfunction interferes with intragenic regulation of mRNA transcription and alternative splicing. *Nucleic Acids Res.* **45**, 5739–5756, https://doi.org/10.1093/nar/gkx163

95 Shirohzu, H., Kubota, T., Kumazawa, A., Sado, T., Chijiwa, T., Inagaki, K. et al. (2002) Three novel DNMT3B mutations in Japanese patients with ICF syndrome. *Am. J. Med. Genet.* **112**, 31–37, https://doi.org/10.1002/ajmg.10658

96 Sandoval, J., Heyn, H., Moran, S., Serra-Musach, J., Pujana, M.A., Bibikova, M. et al. (2011) Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. *Epigenetics* **6**, 692–702, https://doi.org/10.4161/epi.6.6.16196

97 Janssen, A., Colmenares, S.U. and Karpen, G.H. (2018) Heterochromatin: guardian of the genome. *Annu. Rev. Cell Dev. Biol.* **34**, 265–288, https://doi.org/10.1146/annurev-cellbio-100617-062653

98 Felle, M., Hoffmeister, H., Rothammer, J., Fuchs, A., Exler, J.H. and Langst, G. (2011) Nucleosomes protect DNA from DNA methylation *in vivo* and *in vitro*. *Nucleic Acids Res.* **39**, 6956–6969, https://doi.org/10.1093/nar/gkr263

99 Okuwaki, M. and Verreault, A. (2004) Maintenance DNA methylation of nucleosome core particles. *J. Biol. Chem.* **279**, 2904–2912, https://doi.org/10.1074/jbc.M310111200

100 Fuks, F., Hurd, P.J., Deplus, R. and Kouzarides, T. (2003) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res.* **31**, 2305–2312, https://doi.org/10.1093/nar/gkg332

101 Karagianni, P., Amazit, L., Qin, J. and Wong, J. (2008) ICBP90, a novel methyl K9 H3 binding protein linking protein ubiquitination with heterochromatin formation. *Mol. Cell. Biol.* **28**, 705–717, https://doi.org/10.1128/MCB.01598-07

102 Gopalakrishnan, S., Sullivan, B.A., Trazzi, S., Della Valle, G. and Robertson, K.D. (2009) DNMT3B interacts with constitutive centromere protein CENP-C to modulate DNA methylation and the histone code at centromeric regions. *Hum. Mol. Genet.* **18**, 3178–3193, https://doi.org/10.1093/hmg/ddp256

103 Kashiwagi, K., Nimura, K., Ura, K. and Kaneda, Y. (2011) DNA methyltransferase 3b preferentially associates with condensed chromatin. *Nucleic Acids Res.* **39**, 874–888, https://doi.org/10.1093/nar/gkq870

104 Yang, S.M., Kim, B.J., Norwood Toro, L. and Skoultchi, A.I. (2013) H1 linker histone promotes epigenetic silencing by regulating both DNA methylation and histone H3 methylation. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 1708–1713, https://doi.org/10.1073/pnas.1213266110

105 Ren, J., Finney, R., Ni, K., Cam, M. and Muegge, K. (2019) The chromatin remodeling protein Lsh alters nucleosome occupancy at putative enhancers and modulates binding of lineage specific transcription factors. *Epigenetics* **14**, 277–293, https://doi.org/10.1080/15592294.2019.1582275

106 Yu, W., McIntosh, C., Lister, R., Zhu, I., Han, Y., Ren, J. et al. (2014) Genome-wide DNA methylation patterns in LSH mutant reveals de-repression of repeat elements and redundant epigenetic silencing pathways. *Genome Res.* **24**, 1613–1623, https://doi.org/10.1101/gr.172015.114

107 Khamlichi, A.A. and Feil, R. (2018) Parallels between mammalian mechanisms of monoallelic gene expression. *Trends Genet.* **34**, 954–971, https://doi.org/10.1016/j.tig.2018.08.005

108 Hirayama, T. and Yagi, T. (2017) Regulation of clustered protocadherin genes in individual neurons. *Semin. Cell Dev. Biol.* **69**, 122–130

109 Toyoda, S., Kawaguchi, M., Kobayashi, T., Tarusawa, E., Toyama, T., Okano, M. et al. (2014) Developmental epigenetic modification regulates stochastic expression of clustered protocadherin genes, generating single neuron diversity. *Neuron* **82**, 94–108, https://doi.org/10.1016/j.neuron.2014.02.005

110 Jiang, Y., Loh, Y.E., Rajarajan, P., Hirayama, T., Liao, W., Kassim, B.S. et al. (2017) The methyltransferase SETDB1 regulates a large neuron-specific topological chromatin domain. *Nat. Genet.* **49**, 1239–1250, https://doi.org/10.1038/ng.3906

111 Lemaitre, C. and Bickmore, W.A. (2015) Chromatin at the nuclear periphery and the regulation of genome functions. *Histochem. Cell Biol.* **144**, 111–122, https://doi.org/10.1007/s00418-015-1346-y

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