

Chapter 6

Chromosomes and Nuclear Organization in ICF Syndrome

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Abstract The ICF syndrome is a rare, autosomal recessive disorder, often fatal in childhood, and characterized by genetic and clinical heterogeneity. Its most consistent features are reduction in serum immunoglobulin levels, facial anomalies, and cytogenetic defects. ICF is also characterized by abnormal DNA methylation. Significant DNA hypomethylation is present mainly in the classical satellite sequences, the major constituent of the juxtacentromeric heterochromatin of chromosomes 1, 9, and 16. The relationship between DNA methylation defects, altered gene expression, and clinical and phenotypic features in ICF has been the object of intense scrutiny. Although the full pathogenetic picture remains to be elucidated, a number of hypotheses advocating an epigenetic model for this syndrome have been advanced by different research groups. Central to some of these hypotheses is the postulation of a trans-acting regulatory role for the heterochromatin and the suggestion of a possible connection between altered gene expression in ICF and the inappropriate release or recruitment of regulatory complexes by the hypomethylated satellite DNA. This chapter reviews the evidence supporting an association between pathology, large-scale chromatin organization, and nuclear architecture in this enigmatic syndrome.

Introduction

The ICF syndrome (OMIM 242860) is a rare genetic disorder with a distinctive chromosomal phenotype. The acronym ICF was coined more than 20 years ago (Maraschio et al. 1988) to describe a newly identified syndrome characterized

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by immunodeficiency, centromeric instability, and facial anomalies. Cytogenetically ICF is distinguishable because of its variable chromosomal instability and typical structural aberrations, mostly involving the centromeric regions of chromosomes 1 and 16, and to a lesser extent chromosome 9, with the most distinctive feature being the apparent stretching or despiralization of the large blocks of juxtacentromeric—or centromere-adjacent—heterochromatin. Chromosomal anomalies in ICF patients also include homologous and nonhomologous associations of those centromeric regions, multibranching configurations involving one or more of the decondensed chromosomes, nuclear protrusions, micronuclei, and duplications or deletions of whole chromosome arms (Brown et al. 1995; Gimelli et al. 1993; Sawyer et al. 1995a; Tuck-Muller et al. 2000). Those chromosomal abnormalities are almost exclusively encountered in phytohemagglutinin-stimulated lymphocytes. In fibroblasts and Epstein–Barr virus (EBV)-transformed lymphocytes, the cytogenetic manifestations of the syndrome are limited to the occasional elongation of the heterochromatic regions (Maraschio et al. 1989).

The ICF syndrome is often fatal in childhood. The immunodeficiency is typically the cause of early fatalities, and the normal cause of death in ICF patients is infection, usually of the pulmonary or gastrointestinal tract. Although the majority of patients display only humeral immunodeficiency, a considerable number show combined immunodeficiency with additional defective cellular immunity, characterized by a reduction or inversion in the CD4⁺/CD8⁺ T-cell ratio. Thus far, only one patient lacking the immunodeficiency phenotype has been described. Facial anomalies in ICF patients are mild and include hypertelorism, low-set ears, flat nasal bridge, epicanthal folds, and macroglossia. Psychomotor and mental retardation are also often observed in this syndrome. The spectrum of phenotypic and clinical features resulting in the pronounced heterogeneity of the ICF syndrome has been comprehensively covered in a number of expert reviews (Ehrlich 2003; Ehrlich et al. 2008; Hagleitner et al. 2008).

Since its initial description, the enigmatic nature of this syndrome has continued to fascinate scientists worldwide and from different biomedical spheres with the result that, in spite of its rarity—approximately 50 cases reported so far—and the limited availability of study material, published research on various aspects of ICF has flourished over the past 20 years. Although investigations on the ICF syndrome initially seemed almost exclusively the dominion of clinical genetics, cytogenetics, and immunology, with the growing number of case reports and the complexity of the disorder becoming gradually more evident, cross-referencing and the drawing of parallelisms between specific findings in ICF and related aspects of cancer biology, epigenetics, genomics, and developmental biology have become increasingly frequent (Aran et al. 2011; Ehrlich 2009; Ehrlich et al. 2006; Feng and Fan 2009; Martins-Taylor et al. 2012; Toyota and Suzuki 2010; van den Brand et al. 2011). However, notwithstanding the global research efforts and the extensive range of experimental approaches deployed, the full ICF pathogenetic picture remains somehow elusive.

Methylation Defects and Genetic Heterogeneity

Methylation at the 5-position of cytosine within CG dinucleotides is an important epigenetic modification, critical for gene regulation and control of chromatin structure in mammalian cells. One of the hallmark molecular features of the ICF syndrome is defective DNA methylation. Most specifically, ICF patients present with significant constitutive hypomethylation at the classical satellite 2 DNA, the major constituent of the juxtacentromeric heterochromatin at 1qh and 16qh, which is normally hypermethylated in somatic cells. Chromosome 9 juxtacentromeric heterochromatin, which mainly consists of the related satellite 3, also appears to be hypomethylated, although to a lesser extent (Jeanpierre et al. 1993). A small number of other genomic regions have been shown to have significant hypomethylation in ICF syndrome, most notably the centromeric alpha satellites (Miniou et al. 1997a), Alu sequences (Miniou et al. 1997b) and the non-satellite repeats D4Z4 and *NBL2* (Kondo et al. 2000). Single-copy loci showing heterogeneous hypomethylation comprise the imprinted loci D15S9, D15S63, and H19 (Schuffenhauer et al. 1995), and in female ICF cells a number of genes residing on the inactive X chromosome (Hansen et al. 2000; Bourc'his et al. 1999; Miniou et al. 1994).

Initial investigations on the DNA methylation status in ICF were prompted by the observation that chromosome rearrangements seen in ICF cells were reminiscent of the chromosomal changes induced by the undermethylating agent 5-azacytidine (examples in Fig. 6.1). Key cytological evidence implicating DNA hypomethylation in the formation of pericentromeric anomalies in ICF was provided by Ji et al. (1997), who confirmed in a pro-B-cell line the preferential formation at a very high frequency of pericentric rearrangements of chromosome 1—identical to the diagnostic chromosomal aberrations in ICF syndrome—on treatment with the DNA methylation inhibitors 5-azadeoxycytidine and 5-azacytidine.

At the molecular level, the methylation pattern of classical satellite DNA in ICF patients, which mimics an embryonic, undermethylated pattern, was initially shown

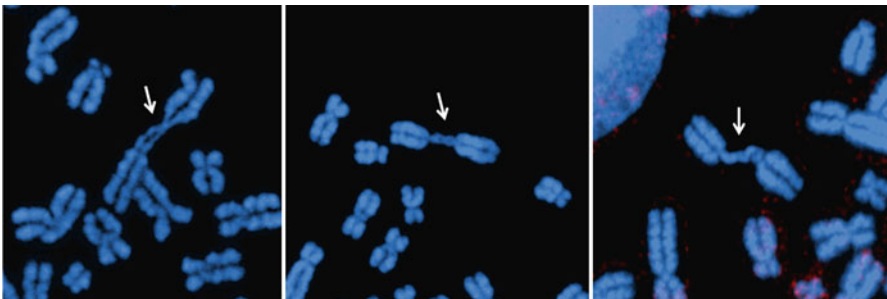


Fig. 6.1 Demethylation by 5-azacytidine induces ICF (immunodeficiency, centromere instability, and facial anomalies syndrome)-like ‘stretching’ of the juxtacentromeric heterochromatin in normal B cells

by restriction endonuclease analysis with methyl-sensitive enzymes (Jeanpierre et al. 1993). Subsequently, Hassan et al. (2001) managed to quantify the extent of the abnormal methylation in ICF by using a bisulfite conversion-based method and showing that the cytosine methylation level of the satellite 2 DNA sequences was almost 70 % in normal lymphoblasts and fibroblasts, compared with only 20 % in ICF cells. In line with previous findings, a paper recently published by the Esteller group (Heyn et al. 2012), reporting whole-genome bisulfite DNA sequencing of an ICF patient, showed that despite a global loss of DNA methylation, the shape of genetic features, such as promoters or CpG islands, is conserved, and the most profound changes occur in inactive heterochromatic regions, satellite repeats, and transposons.

The ICF syndrome—the transmission of which is compatible with an autosomal recessive mode of inheritance—was initially linked to chromosome 20q11.2 by homozygosity mapping (Wijmenga et al. 1998). Subsequently, the DNA methyltransferase 3B gene (*DNMT3B*) was mapped by positional cloning at this chromosomal location and mutations in this gene identified as responsible for the methylation defects observed in ICF (Hansen et al. 1999; Xu et al. 1999). Along with DNMT3A, DNMT3B acts to methylate cytosine residues de novo. The de novo methyltransferases DNMT3A and DNMT3B are essential for normal development, and *Dnmt3b*^{-/-} homozygous mouse embryos present with multiple developmental defects and fail to develop to term, although development appears normal up to E9.5 (Okano et al. 1999).

Dnmt3b colocalizes with Dnmt3a to the pericentromeric heterochromatic regions in murine embryonic stem cells (Bachman et al. 2001). In mammalian cells, DNMT3B has been found to interact with constitutive centromere protein CENP-C to modulate DNA methylation and the histone code at centromeric regions, and its loss has been shown to lead to elevated chromosome misalignment and segregation defects during mitosis and increased transcription of centromeric repeats (Gopalakrishnan et al. 2009). DNMT3B has also been found to associate in heterochromatic regions with HDAC1 and HDAC2, the ATP-dependent chromatin remodeling enzyme hSNF2H, and two components of the histone H3 lysine 9 methylation machinery, namely, HP1 proteins and Suv39h1 (Geiman et al. 2004). In summary, DNMT3B appears to interact with four chromatin-associated enzymatic activities common to transcriptionally repressed, heterochromatic regions of the genome: DNA methyltransferase, histone deacetylase, ATPase, and histone methylase activities.

Mutations of *DNMT3B* in ICF syndrome are heterogeneous. Analysis of 14 patients revealed 11 different mutations, including eight different missense mutations, two nonsense mutations, and a splice site mutation (Wijmenga et al. 2000). Missense mutations of *DNMT3B* in ICF patients occur in or near the catalytic domain of the DNMT3B protein, presumably affecting the normal activity of the enzyme. Nonsense mutations giving rise to a truncated protein always occur as compound heterozygous mutations, highlighting that the DNMT3B protein is essential for life. Most recently, a murine model for ICF syndrome has been engineered by generating *Dnmt3b* mutations in mice (Ueda et al. 2006). Homozygous

mice carrying two missense alleles of *Dnmt3b* show many ICF-like characteristics, and exhibit low body weight, cranial facial anomalies, and T-cell death by apoptosis. They also show hypomethylation of heterochromatin repeat DNA.

Mutations in the *DNMT3B* gene account for approximately 60 % of ICF cases. Wijmenga and collaborators were the first to report a number of ICF patients not carrying mutations in the *DNMT3B* gene (Wijmenga et al. 2000). Similar findings were subsequently published by other research groups (Jiang et al. 2005; Kubota et al. 2004). Jiang et al. (2005) showed that the patients carrying a mutation in the *DNMT3B* gene had alpha satellite methylation patterns comparable to control samples. In contrast, the patients who did not carry mutations in *DNMT3B* exhibited hypomethylation of the alpha satellite DNA as well as classical satellite. These findings led to the proposal of the existence of two distinct types of ICF syndrome with different genetic and epigenetic characteristics, namely, a type 1, in which patients display mutations in the *DNMT3B* gene but have normal alpha satellite methylation, and a type 2, characterized by normal *DNMT3B* and hypomethylation of alpha satellite DNA. There appears to be no genotype–phenotype correlation between patients with and without *DNMT3B* mutations (Hagleitner et al. 2008).

In 2011, by means of homozygosity mapping, whole-exome sequencing and Sanger sequencing on 11 ICF type 2 cases, de Greef and collaborators identified in some of the patients mutations in *ZBTB24*, the zinc-finger- and BTB (bric-a-bric, tramtrack, broad complex)-domain-containing 24, a gene belonging to a large family of transcriptional factors including some members with prominent regulatory roles in hematopoietic development and malignancy (de Greef et al. 2011). *ZBTB24* is ubiquitously expressed and its expression appears to be coregulated with *DNMT3B* during B-cell differentiation. A deletion in the *ZBTB24* gene, resulting in a loss of function of the corresponding protein, was also reported in a consanguineous Lebanese ICF type 2 family with three affected brothers (Chouery et al. 2012). In a *DNMT3B* mutant patient, whole-genome bisulfite DNA sequencing showed that *ZBTB24* harbored a hypermethylated DMR (differentially methylated region) in its promoter, suggesting that inactivating hypermethylation of this gene might contribute to the type 1 disease phenotype (Heyn et al. 2012).

Notwithstanding a great deal of progress with the understanding of the genetic and methylation defects that characterize the ICF syndrome at the molecular level, the causal relationships between *DNMT3B* and/or *ZBTB24* mutations, methylation abnormalities, and the range of ICF phenotypes still needs to be fully clarified.

Original Interphase Studies

Once it became clear that the ICF distinctive ‘chromosomal phenotype’ comprising anomalous mitotic configurations involving mainly chromosomes 1 and 16, and to a lesser extent chromosome 9, combined with an abnormal methylation pattern of the classical satellite DNA, was the hallmark feature of the ICF syndrome, there followed several attempts at investigating the ICF genetic and cytogenetic traits in

interphase as well as in metaphase. Of course, by that time, the introduction of the fluorescence in situ hybridization (FISH) technique to the diagnostic scenario had allowed the reinvention of classical cytogenetics into molecular cytogenetics, opening up the possibility to visualize specific DNA sequences within the nuclear context and allowing the identification of individual chromosomes and the study of chromosomal abnormalities in interphase (Volpi and Bridger 2008).

Maraschio and collaborators published the first study on the interphase behavior of the centromeric heterochromatin of chromosomes 1 and 16 in ICF lymphocytes by means of nonisotopic in situ hybridization, reporting involvement of those chromosomal regions in increased somatic pairing, nuclear protrusions, and micronuclei (Maraschio et al. 1992). Miniou and collaborators carried out the first cytological investigation of DNA methylation defects in ICF using 5-methylcytosine monoclonal antibody to evaluate the methyl content of nuclei and micronuclei (Miniou et al. 1994). They also used alpha and classical satellite probes in single- and dual-color FISH to visualize heterochromatin stretching in interphase. Sawyer and collaborators applied FISH with 'painting' probes for chromosomes 1 and 16 to document the progression of centromeric instability from simple heterochromatin decondensation to the subsequent formation of multibranch configurations, and finally nuclear projections and micronuclei involving both chromosomes 1 and 16 (Sawyer et al. 1995b). Similar interphase cytogenetic investigations were carried out by Stacey et al. (1995). Subsequent FISH experiments using the satellite 2-related probe pHuR 195 confirmed that chromosome fusion in the ICF syndrome occurs only at regions of decondensed centromere-adjacent heterochromatin, and that the alpha satellite repeats, the main component of centromeres, always remain outside the regions of multiradiate chromosome fusions (Sumner et al. 1998).

Gisselsson and collaborators showed, in four ICF lymphoblastoid cell lines, an increased colocalization of the hypomethylated 1qh and 16qh sequences in interphase, abnormal looping of pericentromeric DNA sequences at metaphase, formation of bridges at anaphase, chromosome 1 and 16 fragmentation at the telophase–interphase transition, and, in apoptotic cells, micronuclei with overrepresentation of chromosome 1 and 16 material. Their results suggested that 1qh–16qh associations in interphase can lead to disturbances of mitotic segregation, resulting in micronucleus formation and sometimes apoptosis (Gisselsson et al. 2005). An association between satellite 2 demethylation induced by 5-azacytidine with missegregation of chromosomes 1 and 16 was shown in human somatic cells by Prada et al. (2012).

Overall, the original interphase cytogenetic studies were mostly concerned with tracking the succession of cytological events leading to the distinctive karyotypic features of the ICF syndrome. Accordingly, the main focus of those research endeavors was on the dynamics of chromosomal instability and references to the possible pathogenetic significance of interphase chromosome organization were rare and strictly speculative. However, those initial 'nuclear' observations were fundamental to prepare the experimental and conceptual ground for subsequent studies on the epigenetic impact of nuclear architecture and interphase chromosome organization in the ICF syndrome.

Gene Expression and the Regulation Conundrum

A significant landmark in the sequence of research undertakings aimed at clarifying the connection between methylation defects and gene expression in ICF was a paper published by Ehrlich and collaborators in 2001 (Ehrlich et al. 2001). By means of an oligonucleotide microarray containing approximately 5,600 different human genes, gene expression was analyzed in lymphoblastoid cell lines from six ICF patients carrying different *DNMT3B* mutations. A total of 45 genes were shown to have significant differences in expression in ICF versus control cells. Of the 32 genes described in detail in the paper, a substantial number appears to play a part in lymphogenesis, signal transduction, and apoptosis. Nine genes in particular are implicated in lymphoid cell differentiation or function after V(D)J recombination and are involved in signal transduction or transcriptional control. Despite the differences in gene expression between ICF and control lymphoblastoid cell lines, no differences in methylation at the promoters or 5'-transcribed regions were observed in a sample of genes analyzed by COBRA analysis, leading the authors to suggest that the hypomethylation of classical satellite DNA sequences in ICF syndrome might result in a decreased 'heterochromatinization' that affects the regulation of genes elsewhere in the genome.

Global expression profiling of three patients with *DNMT3B* mutations was published by Jin et al. (2008). This study generated an impressive list of nearly 800 genes appearing to be up- or downregulated in ICF cells, a large number of which are involved with immune function, development, and neurogenesis. Detailed DNA methylation mapping on a subset of deregulated genes revealed that a fraction of them are typically methylated at low levels in normal cells and lose their methylation in ICF cells. By chromatin immune precipitation the authors also showed that histone modification patterns at affected promoters were dramatically altered, demonstrating for the first time that loss of DNMT3B function in ICF can lead to significant hypomethylation of nonrepetitive regions of the genome and alterations in the histone code. They also observed loss of binding of the SUZ12 component of the PRC2 polycomb repression complex and DNMT3B to derepressed genes, including a number of homeobox genes critical to the immune system and craniofacial development.

Heterochromatic genes have also been reported to undergo epigenetic changes and escape silencing in ICF (Brun et al. 2011); however, the precise mechanism driving their transcription is unclear. Indeed, although DNA hypomethylation was found in all heterochromatic genes and in all patients analyzed, gene expression was restricted to some genes and every patient had his or her own group of activated genes. Surprisingly, heterochromatic genes—either active or inactive—appeared to be associated with histone modifications typical of inactive chromatin. Hypomethylation of subtelomeric regions, associated with aberrant transcription and advanced replication timing of these sequences, has also been described in ICF (Yehezkel et al. 2008).

All together, these expression studies have succeeded in shedding important light on the extent and nature of gene deregulation in ICF resulting from the combined

effect of DNA hypomethylation and alterations in the histone code. However, even allowing for prediction of alterations in relevant downstream targets of misexpressed transcriptional regulators, it is obvious that the full ICF epigenetic picture is not yet complete. In summary, on the basis of what has been published so far, and taking also in consideration recent evidence obtained by whole-genome bisulfite DNA sequencing of a DNMT3B mutant patient showing that, despite global loss of genomic DNA methylation, transcriptional active loci and rRNA repeats escape hypomethylation and overall the shape of genetic features such as promoters and CpG islands is conserved (Heyn et al. 2012), it is apparent that the relationship between methylation defects, altered gene expression, and clinical and phenotypic features in ICF needs further investigation.

The Nuclear Architecture Perspective

Over the years a number of hypotheses advocating an epigenetic model for the ICF syndrome have been advanced by different research groups. In particular, possible mechanisms linking altered gene expression to the hypomethylation of pericentromeric heterochromatin have been postulated, generally envisaging inappropriate release or recruitment of regulatory complexes by the hypomethylated satellite DNA, affecting the transacting regulatory properties of the heterochromatin (Hassan et al. 2001; Hansen et al. 1999; Xu et al. 1999; Ehrlich et al. 2001; Bickmore and van der Maarel 2003).

Although so far only a handful of investigations have attempted to test those hypotheses, some interesting findings have already emerged. For example, two publications have independently reported repositioning of the chromosome 1 juxtacentromeric heterochromatin domain to a more interior location within the nuclear volume in ICF lymphocytes (Dupont et al. 2012; Jefferson et al. 2010). More specifically, microscope observations on a cell-by-cell basis, and comparative measurements of the distance between chromosome 1 juxtacentromeric heterochromatin and the nuclear rim carried out in our laboratory, have revealed that the extent of heterochromatin association with the extreme nuclear periphery is reduced in ICF cells (both type 1 and type 2), suggesting its specific repositioning to a more internal location within the nuclear space (Jefferson et al. 2010). Dupont and collaborators have independently confirmed repositioning of the chromosome 1 juxtacentromeric heterochromatin toward the nuclear interior by means of three-dimensional (3D) FISH and confocal microscopy analysis on type 2 patient cells (Dupont et al. 2012). Based on the differential distribution of early and late-replicating chromatin within the nuclear space (Ferreira et al. 1997; Sadoni et al. 1999), these findings on the relocation of the juxtacentromeric heterochromatin away from the extreme nuclear periphery to a more internal position appear to be in line with the previously reported data on the advanced replication of the hypomethylated satellite 2 in ICF (Hassan et al. 2001).

Although its role in actively regulating gene expression remains unproven, the nuclear periphery is generally considered a transcriptionally silent 'address' within

the nuclear volume, characterized by poor gene density (Croft et al. 1999; Shopland et al. 2006; Tanabe et al. 2002) and a high concentration of nontranscribed sequences (Scheuermann et al. 2004). Interestingly, a case of functional repositioning of the chromosome 1 juxtacentromeric heterochromatin within the nuclear volume had been reported previously by Barki-Celli et al. (2005), who found that the 1q12 heterochromatin domain location within the nuclear volume was different when resting B cells were compared to B cells treated with trichostatin A (TSA), a histone deacetylase inhibitor, with the induced hyperacetylation shown to cause a significant reduction in the percentage of 1q12 signals associated with the nuclear periphery, probably by interfering with the establishment of ‘epigenetic codes’ required for localizing 1q12 to the nuclear membrane, as suggested by the authors.

Long-range heterochromatin–gene associations have gained recognition as a potential novel epigenetic mechanism of gene regulation in mammalian cells. So far, a correlation between gene silencing and localization to transcriptionally repressive heterochromatic compartments has been reported in mouse cycling lymphocytes (Brown et al. 1997, 1999; Grogan et al. 2001), human and mouse erythroid cells (Francastel et al. 1999, 2001; Schubeler et al. 2000), and retinoblastoma cells (Bartova et al. 2002). Replacement of the endogenous β -globin locus control region (LCR) with an ectopic regulatory element, the IgH LCR, was shown to induce looping of the globin locus out of its chromosome territory and bring it closer to the repressive centromeric heterochromatin compartment by Ragozy et al. (2003). A link between centromeric recruitment and establishment of allelic exclusion at the immunoglobulin heavy-chain gene in mouse B cells was also reported (Roldan et al. 2005). In many instances, uncertainty remains whether nonrandom positioning and association of genes with specific nuclear compartments are the cause or the consequence of gene function, although the general consensus points toward an interactive model in which the functional potential of a locus facilitates its association with a nuclear compartment, which in turn influences the functional properties of the locus (Misteli 2004). Accordingly, recruitment to heterochromatin domains may help to stabilize the inactive state, rather than actively promoting silencing.

In our laboratory, we were interested in understanding if the decondensation of the juxtacentromeric heterochromatin, as observed in metaphase, and general chromosomal instability reported in ICF patients, corresponded to changes in the three-dimensional properties of the heterochromatin in interphase, our working hypothesis being that disruption in the heterochromatin spatial configuration might interfere with transcriptional silencing and be indirectly responsible for the changes in gene expression accounting for the symptoms of ICF. Accordingly, as well as the heterochromatin intranuclear positioning, we analyzed and compared, in patients and controls, the large-scale organization of the juxtacentromeric heterochromatin of chromosome 1 and the intranuclear positioning of a set of genes—four from chromosome 1 and one as a control from chromosome 6, on which we had concurrently conducted expression and methylation analysis—and their colocalization with the juxtacentromeric heterochromatin of chromosome 1. Examples of our findings are presented in Figs. 6.2 and 6.3. In contrast to the consensual view that decondensation and stretching of the juxtacentromeric heterochromatic blocks—similar to that

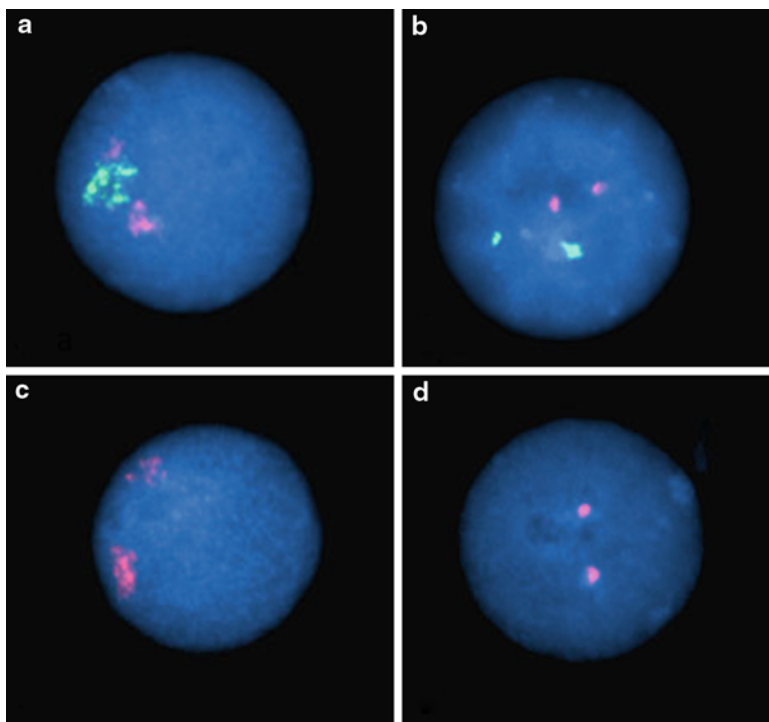


Fig. 6.2 The intranuclear positioning and large-scale organization of the juxtacentromeric heterochromatin is altered in ICF B cells. Chromosome 1 heterochromatin is visualized by two-dimensional (2D) fluorescence in situ hybridization (FISH) with the classical satellite probes D1Z1 (*red*) in nuclei from two controls (**a**, **c**), an ICF type 1 patient (**b**), and an ICF type 2 patient (**d**). Chromosome 16 heterochromatin is visualized by cohybridization with D16Z3 (*green*) (**a**, **b**)

observed in ICF cells in metaphase—could also be expected in interphase, we found that in ICF nuclei the juxtacentromeric heterochromatin appears more compactly shaped. We also found that for two of the loci analyzed—*CNN3* and *RGS1*—the extent of gene–heterochromatin colocalization was significantly reduced in ICF cells, in which these genes are overexpressed but present no changes in promoter methylation (Jefferson et al. 2010).

Our combined findings on the altered large-scale organization and intranuclear positioning of chromosome 1 juxtacentromeric heterochromatin in ICF are particularly significant in the light of the mounting experimental evidence suggesting chromosome band 1q12, which corresponds to the juxtacentromeric heterochromatin of chromosome 1, to be the core of a nuclear domain with functional significance. Earlier investigations showed physical association of this genomic region with the human polycomb group complex, a repressor of the homeotic gene expression (Saurin et al. 1998), and also with the oncogenic transcriptional regulator TLX1/HOX11 in leukemic T cells (Heidari et al. 2006). Most relevantly, in ICF cells the

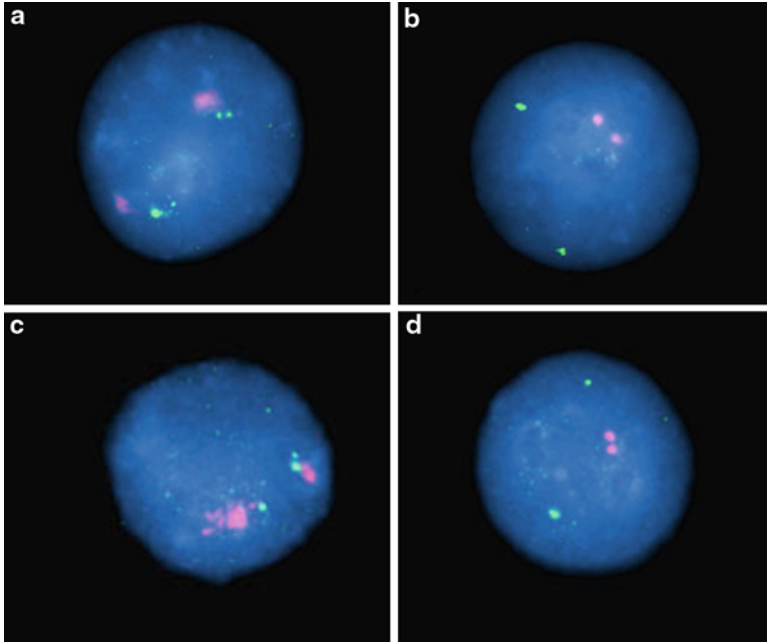


Fig. 6.3 The extent of interphase gene–heterochromatin colocalization for the two loci RGS1 (*green*) (a, b) and CNN3 (*green*) (c, d) is significantly reduced in ICF cells (b, d), in which they are significantly overexpressed, when compared to respective controls (a, c). Chromosome 1 heterochromatin is visualized in *red* by hybridization with D1Z1 in all four images

subcellular distribution of HP1 proteins is altered and the 1qh satellite DNA is associated in G₂ with a giant HP1–PML nuclear body (Luciani et al. 2005, 2006).

A large-scale effect of DNA hypomethylation on the organization of chromosome ‘territories’ within the nuclear volume in ICF was proposed by Matarazzo et al. (2007) on the basis of their findings on repositioning of misexpressed genes located in the pseudo-autosomal region 2 (PAR2) of the X and Y chromosomes. As DNA hypomethylation appears to cause changes in the interphase organization of the inactive chromosome X territory that extends far beyond the genes which are immediately subject to hypomethylation and transcriptional activation, they suggest that it is plausible that elsewhere in the ICF genome, altered chromosome territory organization encompassing extended regions around hypomethylated sequences may allow for the inappropriate transcriptional activation of relocated genes if the right transcription factor environment is available.

Finally, DNA replication appears to be globally affected in ICF cells. Gartler and collaborators (Hansen et al. 2000) indicated advanced replication time as a major determinant of escape from silencing for genes on the inactive X and Y chromosomes they had identified in ICF syndrome. They also showed that satellite 2 replication in ICF is advanced compared with that in normal cells (Hassan et al. 2001). Yehezkel and collaborators reported that hypomethylation of subtelomeric regions

was associated in ICF with advanced telomere replication timing (Yehezkel et al. 2008). More recently, the De Sario group reported that in ICF cells heterochromatic genes replicate earlier in the S-phase, global replication fork speed is higher, and S-phase is shorter, and suggested that these replication defects may result from chromatin changes which modify DNA accessibility to the replication machinery (Lana et al. 2012).

From what has been published so far on different aspects of interphase organization in ICF cells, a distinctive ICF ‘nuclear phenotype’ has begun to emerge in which both local and global changes in the chromatin large-scale organization and positioning, together with changes in chromatin proteins distribution, appear mechanistically linked to the deregulation or impairment of fundamental nuclear functions, including chromosome condensation and segregation, DNA replication, and transcription.

Conclusion

The complexity of ICF, in particular the combination of phenotypic variability and genetic heterogeneity that characterizes it, has intrigued geneticists and cell biologists since this syndrome was initially described more than 20 years ago. Indeed, irrespective of its rare occurrence, ICF has been the object of numerous and diverse studies that have yielded interesting insights into its pathogenesis and prompted a substantial amount of speculation on the relationship between methylation defects and the ICF phenotypic spectrum. Up to now only a small number of investigations have attempted to study the ICF syndrome from a nuclear architecture perspective. However, findings that have emerged so far appear to support—cautiously but consistently—a role for large-scale chromatin organization in the interphase nucleus as a structural and functional intermediary between DNA hypomethylation and altered gene expression in ICF, suggesting the existence of additional layers of epigenetic complexity in this syndrome and warranting further investigations.

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Some of the images used in Figs. 6.1, 6.2, and 6.3 were reproduced from Jefferson et al. (2010), *PLoS ONE* 5(6):e11364, an open-access article distributed under the terms of the Creative Commons Attribution License.

References

- Aran D et al (2011) Replication timing-related and gene body-specific methylation of active human genes. *Hum Mol Genet* 20(4):670–680
- Bachman KE, Rountree MR, Baylin SB (2001) Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. *J Biol Chem* 276(34):32282–32287
- Barki-Celli L et al (2005) Differences in nuclear positioning of 1q12 pericentric heterochromatin in normal and tumor B lymphocytes with 1q rearrangements. *Genes Chromosomes Cancer* 43(4):339–349

- Bartova E et al (2002) Nuclear structure and gene activity in human differentiated cells. *J Struct Biol* 139(2):76–89
- Bickmore WA, van der Maarel SM (2003) Perturbations of chromatin structure in human genetic disease: recent advances. *Hum Mol Genet* 2:5
- Bourc'his D et al (1999) Abnormal methylation does not prevent X inactivation in ICF patients. *Cytogenet Cell Genet* 84(3–4):245–252
- Brown DC et al (1995) ICF syndrome (immunodeficiency, centromeric instability and facial anomalies): investigation of heterochromatin abnormalities and review of clinical outcome. *Hum Genet* 96(4):411–416
- Brown KE et al (1997) Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* 91(6):845–854
- Brown KE et al (1999) Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. *Mol Cell* 3(2):207–217
- Brun ME et al (2011) Heterochromatic genes undergo epigenetic changes and escape silencing in immunodeficiency, centromeric instability, facial anomalies (ICF) syndrome. *PLoS One* 6(4):e19464
- Chouery E et al (2012) A novel deletion in ZBTB24 in a Lebanese family with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. *Clin Genet* 82(5):489–493
- Croft JA et al (1999) Differences in the localization and morphology of chromosomes in the human nucleus. *J Cell Biol* 145(6):1119–1131
- de Greef JC et al (2011) Mutations in ZBTB24 are associated with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. *Am J Hum Genet* 88(6):796–804
- Dupont C et al (2012) 3D position of pericentromeric heterochromatin within the nucleus of a patient with ICF syndrome. *Clin Genet* 82(2):187–192
- Ehrlich M (2003) The ICF syndrome, a DNA methyltransferase 3B deficiency and immunodeficiency disease. *Clin Immunol* 109(1):17–28
- Ehrlich M (2009) DNA hypomethylation in cancer cells. *Epigenomics* 1(2):239–259
- Ehrlich M et al (2001) DNA methyltransferase 3B mutations linked to the ICF syndrome cause dysregulation of lymphogenesis genes. *Hum Mol Genet* 10(25):2917–2931
- Ehrlich M, Jackson K, Weemaes C (2006) Immunodeficiency, centromeric region instability, facial anomalies syndrome (ICF). *Orphanet J Rare Dis* 1:2
- Ehrlich M et al (2008) ICF, an immunodeficiency syndrome: DNA methyltransferase 3B involvement, chromosome anomalies, and gene dysregulation. *Autoimmunity* 41(4):253–271
- Feng J, Fan G (2009) The role of DNA methylation in the central nervous system and neuropsychiatric disorders. *Int Rev Neurobiol* 89:67–84
- Ferreira J et al (1997) Spatial organization of large-scale chromatin domains in the nucleus: a magnified view of single chromosome territories. *J Cell Biol* 139(7):1597–1610
- Francastel C et al (1999) A functional enhancer suppresses silencing of a transgene and prevents its localization close to centromeric heterochromatin. *Cell* 99(3):259–269
- Francastel C, Magis W, Groudine M (2001) Nuclear relocation of a transactivator subunit precedes target gene activation. *Proc Natl Acad Sci U S A* 98(21):12120–12125
- Geiman TM et al (2004) DNMT3B interacts with hSNF2H chromatin remodeling enzyme, HDACs 1 and 2, and components of the histone methylation system. *Biochem Biophys Res Commun* 318(2):544–555
- Gimelli G et al (1993) ICF syndrome with variable expression in sibs. *J Med Genet* 30(5):429–432
- Gisselsson D et al (2005) Interphase chromosomal abnormalities and mitotic missegregation of hypomethylated sequences in ICF syndrome cells. *Chromosoma (Berl)* 114(2):118–126
- Gopalakrishnan S et al (2009) DNMT3B interacts with constitutive centromere protein CENP-C to modulate DNA methylation and the histone code at centromeric regions. *Hum Mol Genet* 18(17):3178–3193
- Grogan JL et al (2001) Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. *Immunity* 14(3):205–215

- Hagleitner MM et al (2008) Clinical spectrum of immunodeficiency, centromeric instability and facial dysmorphism (ICF syndrome). *J Med Genet* 45(2):93–99
- Hansen RS et al (1999) The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc Natl Acad Sci U S A* 96(25):14412–14417
- Hansen RS et al (2000) Escape from gene silencing in ICF syndrome: evidence for advanced replication time as a major determinant. *Hum Mol Genet* 9(18):2575–2587
- Hassan KM et al (2001) Satellite 2 methylation patterns in normal and ICF syndrome cells and association of hypomethylation with advanced replication. *Hum Genet* 109(4):452–462
- Heidari M et al (2006) The nuclear oncoprotein TLX1/HOX11 associates with pericentromeric satellite 2 DNA in leukemic T-cells. *Leukemia* 20(2):304–312
- Heyn H et al (2012) Whole-genome bisulfite DNA sequencing of a DNMT3B mutant patient. *Epigenetics* 7:6
- Jeanpierre M et al (1993) An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. *Hum Mol Genet* 2(6):731–735
- Jefferson A et al (2010) Altered intra-nuclear organisation of heterochromatin and genes in ICF syndrome. *PLoS One* 5(6):e11364
- Ji W et al (1997) DNA demethylation and pericentromeric rearrangements of chromosome 1. *Mutat Res* 379(1):33–41
- Jiang YL et al (2005) DNMT3B mutations and DNA methylation defect define two types of ICF syndrome. *Hum Mutat* 25(1):56–63
- Jin B 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(5):690–709
- Kondo T et al (2000) Whole-genome methylation scan in ICF syndrome: hypomethylation of non-satellite DNA repeats D4Z4 and NBL2. *Hum Mol Genet* 9(4):597–604
- Kubota T et al (2004) ICF syndrome in a girl with DNA hypomethylation but without detectable DNMT3B mutation. *Am J Med Genet A* 1(3):290–293
- Lana E et al (2012) DNA replication is altered in immunodeficiency centromeric instability facial anomalies (ICF) cells carrying DNMT3B mutations. *Eur J Hum Genet* 29(10):41
- Luciani JJ et al (2005) Subcellular distribution of HP1 proteins is altered in ICF syndrome. *Eur J Hum Genet* 13(1):41–51
- Luciani JJ et al (2006) PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. *J Cell Sci* 119(pt 12):2518–2531
- Maraschio P et al (1988) Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial anomalies: the ICF syndrome. *J Med Genet* 25(3):173–180
- Maraschio P et al (1989) Differential expression of the ICF (immunodeficiency, centromeric heterochromatin, facial anomalies) mutation in lymphocytes and fibroblasts. *J Med Genet* 26(7):452–456
- Maraschio P et al (1992) Interphase cytogenetics of the ICF syndrome. *Ann Hum Genet* 56(pt 3):273–278
- Martins-Taylor K et al (2012) Role of DNMT3B in the regulation of early neural and neural crest specifiers. *Epigenetics* 7:1
- Matarazzo MR et al (2007) Chromosome territory reorganization in a human disease with altered DNA methylation. *Proc Natl Acad Sci U S A* 104(42):16546–16551
- Miniou P et al (1994) Abnormal methylation pattern in constitutive and facultative (X inactive chromosome) heterochromatin of ICF patients. *Hum Mol Genet* 3(12):2093–2102
- Miniou P et al (1997a) Alpha-satellite DNA methylation in normal individuals and in ICF patients: heterogeneous methylation of constitutive heterochromatin in adult and fetal tissues. *Hum Genet* 99(6):738–745
- Miniou P et al (1997b) Undermethylation of Alu sequences in ICF syndrome: molecular and in situ analysis. *Cytogenet Cell Genet* 77(3–4):308–313
- Misteli T (2004) Spatial positioning: a new dimension in genome function. *Cell* 119(2):153–156

- Okano M et al (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99(3):247–257
- Prada D et al (2012) Satellite 2 demethylation induced by 5-azacytidine is associated with missegregation of chromosomes 1 and 16 in human somatic cells. *Mutat Res* 729(1–2):100–105
- Ragoczy T et al (2003) A genetic analysis of chromosome territory looping: diverse roles for distal regulatory elements. *Chromosome Res* 11(5):513–525
- Roldan E et al (2005) Locus ‘decontraction’ and centromeric recruitment contribute to allelic exclusion of the immunoglobulin heavy-chain gene. *Nat Immunol* 6(1):31–41
- Sadoni N et al (1999) Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments. *J Cell Biol* 146(6):1211–1226
- Saurin AJ et al (1998) The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain. *J Cell Biol* 142(4):887–898
- Sawyer JR et al (1995a) Chromosome instability in ICF syndrome: formation of micronuclei from multibranching chromosomes 1 demonstrated by fluorescence in situ hybridization. *Am J Med Genet* 56(2):203–209
- Sawyer JR et al (1995b) Centromeric instability of chromosome 1 resulting in multibranching chromosomes, telomeric fusions, and “jumping translocations” of 1q in a human immunodeficiency virus-related non-Hodgkin’s lymphoma. *Cancer (Phila)* 76(7):1238–1244
- Scheuermann MO et al (2004) Topology of genes and nontranscribed sequences in human interphase nuclei. *Exp Cell Res* 301(2):266–279
- Schubeler D et al (2000) Nuclear localization and histone acetylation: a pathway for chromatin opening and transcriptional activation of the human beta-globin loci. *Genes Dev* 14(8):940–950
- Schuffenhauer 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(5):562–571
- Shopland LS et al (2006) Folding and organization of a contiguous chromosome region according to the gene distribution pattern in primary genomic sequence. *J Cell Biol* 174(1):27–38
- Stacey M, Bennett MS, Hulten M (1995) FISH analysis on spontaneously arising micronuclei in the ICF syndrome. *J Med Genet* 32(7):502–508
- Sumner AT, Mitchell AR, Ellis PM (1998) A FISH study of chromosome fusion in the ICF syndrome: involvement of paracentric heterochromatin but not of the centromeres themselves. *J Med Genet* 35(10):833–835
- Tanabe H et al (2002) Non-random radial arrangements of interphase chromosome territories: evolutionary considerations and functional implications. *Mutat Res* 504(1–2):37–45
- Toyota M, Suzuki H (2010) Epigenetic drivers of genetic alterations. *Adv Genet* 70:309–323
- Tuck-Muller CM et al (2000) DNA hypomethylation and unusual chromosome instability in cell lines from ICF syndrome patients. *Cytogenet Cell Genet* 89(1–2):121–128
- Ueda Y et al (2006) Roles for Dnmt3b in mammalian development: a mouse model for the ICF syndrome. *Development (Camb)* 133(6):1183–1192
- van den Brand M et al (2011) Angiosarcoma in a patient with immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome. *Am J Med Genet A* 3:622–625
- Volpi EV, Bridger JM (2008) FISH glossary: an overview of the fluorescence in situ hybridization technique. *Biotechniques* 45(4):385–386, 388, 390, passim
- Wijmenga C et al (1998) Localization of the ICF syndrome to chromosome 20 by homozygosity mapping. *Am J Hum Genet* 63(3):803–809
- Wijmenga C et al (2000) Genetic variation in ICF syndrome: evidence for genetic heterogeneity. *Hum Mutat* 16(6):509–517
- Xu GL et al (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature (Lond)* 402(6758):187–191
- Yehezkel S et al (2008) Hypomethylation of subtelomeric regions in ICF syndrome is associated with abnormally short telomeres and enhanced transcription from telomeric regions. *Hum Mol Genet* 17(18):2776–2789