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Genomic Imprinting: CTCF Protects the Boundaries

Dispatch

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The DNA-binding protein CTCF, which acts as a chromatin 'insulator', regulates imprinting of the mammalian *Igf2* and *H19* genes in a methylation-sensitive manner. It has now been shown that CTCF is also required for protection against *de novo* methylation of the differentially methylated domain of *H19* in the female germline.

Genomic imprinting is a mode of inheritance whereby only one parental allele is expressed, while the other is silenced. DNA methylation plays a role in this gene silencing. In humans and mice, the *Igf2* and *H19* genes are reciprocally imprinted such that *Igf2* is expressed from the paternal allele and *H19* from the maternal allele. Transcription of these genes is controlled by a set of shared enhancers downstream of *H19* and a differentially methylated domain (DMD) upstream of *H19*. This DMD contains binding sites for the protein CCCTC binding factor (CTCF), which acts as a chromatin insulator (Figure 1A). On the maternally inherited chromosome, CTCF binds to the unmethylated DMD, creating a chromatin insulator which prevents the *Igf2* promoter from gaining access to the downstream enhancers. On the paternally inherited allele, the DMD is methylated, which blocks CTCF binding; this lack of CTCF binding presumably inactivates the insulator, allowing the promoter of the paternal *Igf2* allele to interact with the downstream enhancers [1,2].

The *H19* DMD is known as an 'imprinting centre' which carries a 'germ line imprint', as the differential methylation is set up in the parental germ cells and then maintained throughout development. Other differentially methylated regions, such as those found in the *Igf2* gene, are significantly reprogrammed during development. The mechanisms for the reprogramming and setting of imprints in the *Igf2–H19* region are still poorly understood, but there is now evidence implicating CTCF in this process.

In independent studies, two groups [3,4] have mutated CTCF binding sites in the *H19* DMD and demonstrated that the maternal *H19* DMD acquires methylation during post implantation development in the absence of CTCF binding. These studies indicate that binding of CTCF to the DMD is necessary to maintain the unmethylated state of the maternal allele in somatic cells. More recently, the results of a series of *in vitro* transfection assays, reported in an upcoming issue of *Current Biology* [5], have confirmed that CTCF binding sites protect themselves and adjacent sequences against methylation. From these thought-provoking results we can conclude, not only that CTCF binding is sensitive to methylation, but that methylation itself is dependent on CTCF binding.

Fedoriw *et al.* [6] have now taken this one stage further by removing CTCF from the equation altogether. Using an approach based on RNA interference (RNAi) in transgenic mice, they selectively ablated CTCF expression in the oocyte and found increased methylation in the *H19* DMD associated with substantial loss of CTCF protein. Their results indicate that CTCF is required for establishment, as well as maintenance, of differential methylation and imprinting of *H19*. They also noted that CTCF-deficient oocytes have decreased developmental competence, suggesting that CTCF is important for normal preimplantation development.

These recent studies [3–6] clearly show that CTCF is important for the regulation of differential methylation, as well as imprinted expression of the *Igf2–H19* region. However, Schoenherr *et al.* [4] found that, when the CTCF binding sites in the *H19* DMD were mutated, methylation at that DMD remained unchanged in oocytes and early blastocysts. This is contrary to the findings of Fedoriw *et al.* [6] who found that oocytes gain methylation when CTCF is downregulated by RNAi. The implication is that CTCF regulates *H19* DMD methylation by different mechanisms in germline and somatic lineages.

The challenge now is to work out what the results from these two different approaches tell us about CTCF and the boundary element at the *H19* DMD. While one approach investigated the effects of CTCF binding specifically to the *H19* DMD, the other investigated the wider role of CTCF in the oocyte genome. It appears that CTCF binding protects the DMD from *de novo* methylation in somatic cells by a direct mechanism, but the increase in methylation observed in CTCF-deficient oocytes may reflect another function of CTCF and boundaries.

Insulators or boundary elements are DNA sequences that act as neutral barriers against the influence of neighbouring elements and separate the genome into independent functional domains [7]. Their main functions are preventing external enhancers from accessing a locus and blocking spread of inactive chromatin [8]. A popular theory is that such mechanisms involve higher-order chromatin structures which enable CTCF boundary elements to interact so as to form loop domains [9,10]. Other studies have shown that CTCF can be associated with the so-called 'nuclear matrix', suggesting that it might be involved in nuclear organisation [11,12]. The *H19* DMD itself has been shown not to associate with the nuclear matrix [13], but CTCF may still mediate matrix binding and long-range chromatin interactions in conjunction with known matrix attachment regions within the locus.

We have proposed that the imprinting of the *Igf2–H19* region is maintained on the maternal allele by interaction between the *H19* DMD and differentially

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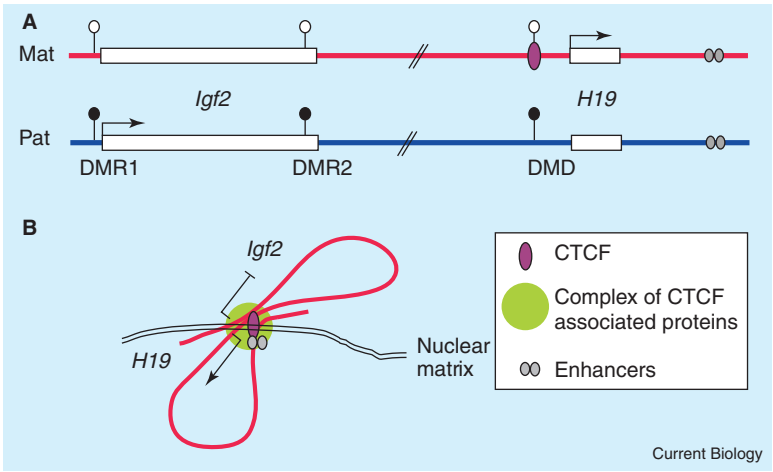

Figure 1. Regulation of imprinting by CTCF.

(A) A diagram illustrating CTCF-dependent regulation of the *Igf2–H19* locus, including the differentially methylated regions, DMR1 and DMR2, of *Igf2* and the germline differentially methylated domain (DMD) at H19. Filled and open lollipops represent methylated and unmethylated differentially methylated regions, respectively. On the unmethylated maternal allele, binding of CTCF (purple oval) blocks the access of the *Igf2* promoter to the enhancers (small circles), which consequently can only activate the *H19* promoter. On the paternal allele, the methylated *H19* DMD does not bind CTCF, allowing expression of Igf2. (B) A general model of the maternal *Igf2–H19* region, showing an example of a higher-order chromatin structure where CTCF binds at one or more sites but can protect against methylation elsewhere. This structure may be associated with the nuclear matrix and involve proteins in addition to CTCF. Tissue-specific variation in higher-order structure could be due to different CTCF-dependent *cis* elements forming the base of the loop or different proteins acting in *trans*.

methylated regions of *Igf2*, based on our observation that deletion of the *H19* DMD leads to hierarchical methylation changes at the *Igf2* differentially methylated regions [14]. We also showed that, while deletion of the entire *H19* DMD protects against methylation of the maternal *Igf2* allele in somatic cells, it has no effect on methylation in oocytes [14]. We found no evidence of CTCF binding to *Igf2 in vivo* (unpublished data), so the recent results discussed above [3–6] suggest that CTCF binding at the *H19* DMD may regulate chromatin structure of the whole domain, preventing methylation of the maternal chromosome.

These findings do not exclude the possibility that, instead of creating a loop, CTCF binding creates a barrier that prevents the spread of inactive chromatin and/or DNA methylation. The barrier and loop domain models — which are not necessarily mutually exclusive — could be tested by investigating methylation and chromatin changes in the different systems, to see if they occur uniformly throughout the locus or are localised at specific regulatory elements.

We must now consider the possibility that imprinting at *H19* depends partly on higher-order chromatin structures, and that CTCF is a major component necessary for establishing these structures and therefore imprinting of the locus (Figure 1B). It is possible that the *H19* DMD becomes methylated as a default state, as a result of intrinsic properties of the DMD sequence or surrounding region, and in the paternal germline methylation of the DMD occurs simply because CTCF does not bind. In the female germline and on the maternal chromosome in somatic cells, CTCF prevents this default methylation, presumably by excluding DNA methyltransferases from the locus. CTCF regulated higher-order structures could vary in different cell types; indeed, it has been shown that matrix attachment at the *Igf2–H19* locus is tissue specific as well as parent-of-origin specific [13].

If the higher-order chromatin structure in oocytes is different from that in somatic cells, additional protective mechanisms may be in place to maintain the chromatin structure and protect against *de novo* methylation in oocytes when CTCF binding sites in the *H19* DMD are mutated or deleted. The lack of methylation protection with global loss of CTCF in the oocyte might indicate that another CTCF-mediated boundary element is involved in setting up the secondary structure and protects against methylation along the locus. CTCF binding sites have been described and characterised for insulator activity *in vitro* further downstream of the *H19* gene [14], suggesting that there are additional insulators at this locus.

Additional support for the view that *cis* and *trans* acting factors required for protection against methylation in the maternal germline comes from the recent results of Cerrato *et al.* [16], who found that the oocytes in a mouse line carrying a cytogenetic inversion distal to the *H19* gene also gain methylation at the *H19* DMD. Thus, there are sequences as far as 30 kilobases downstream of *H19* involved in protection from methylation in the maternal germ line. A global loss of CTCF would not only disrupt the insulator at *H19*, but would potentially affect any other CTCF-dependent insulator or regulatory element in the region, leading to the aberrant methylation seen by Fedoriw *et al.* [6]. The fact that the CTCF-deficient oocytes also show a decrease in developmental competence might be indicative of more wide-scale changes in nuclear organisation. Although Fedoriw *et al.* [6] assayed methylation levels at other loci and found no significant changes, none of the reported loci are known to be regulated by CTCF.

Alternatively, CTCF may positively or negatively regulate other factors directly involved in the protection from or targeting of methylation to the DMD. One such factor might be the CTCF paralogue known as 'Brother of the Regulator of Imprinted sites' (BORIS). BORIS recognises the same DNA binding sites as CTCF, but it is expressed exclusively in the testis [17]. This suggests a model in which BORIS, rather than CTCF, binds to the *H19* DMD in the paternal germline, allowing methylation of the region. It should be interesting to look for

changes in BORIS expression or localisation in CTCF-deficient oocytes.

Fedoriw *et al.* [6] used the powerful RNAi technique to disrupt the ubiquitous regulatory protein CTCF in the germline, thereby uncovering yet another function of CTCF. They have shown that protection of the *H19* DMD in the oocytes clearly depends on CTCF, while earlier results suggest this protection does not occur as a result of CTCF binding directly to the DMD. Determining the nature of this protection mechanism and the role of CTCF in the oocyte will be important in the field of genomic imprinting, and may also give insight into other chromatin based regulatory mechanisms.

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