PERSPECTIVES: MOLECULAR BIOLOGY

Mothers Setting Boundaries

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Mammals undergo the unique process of genomic imprinting. In this process, a gene on one chromosome is silenced, whereas its allele on the other chromosome is expressed. There are several ways to silence genes, the best characterized of which is methylation, the addition of methyl groups to cytosine residues of cytosine-guanosine (CG) dinucleotides in the DNA. Although the silenced allele of some imprinted genes is methylated, the silenced allele of other imprinted genes is not methylated, suggesting that there are other methods for repressing expression of imprinted genes. Now, a flurry of papers including those from the Tilghman (*1*) and Felsenfeld (*2*) groups appearing in a recent issue of *Nature*, indicate that genes can be imprinted through chromatin boundary elements (insulators) that shield the promoter of an imprinted gene from enhancers that boost its expression.

The small number of genes affected by the curious phenomenon of imprinting exhibit allelic expression differences that depend upon parental origin. One of the earliest recognized hallmarks of imprinted genes is that they reside in clusters. Importantly, these clusters are conserved between mouse and human, supporting the hypothesis that the clustering of imprinted genes is essential to their regulation.

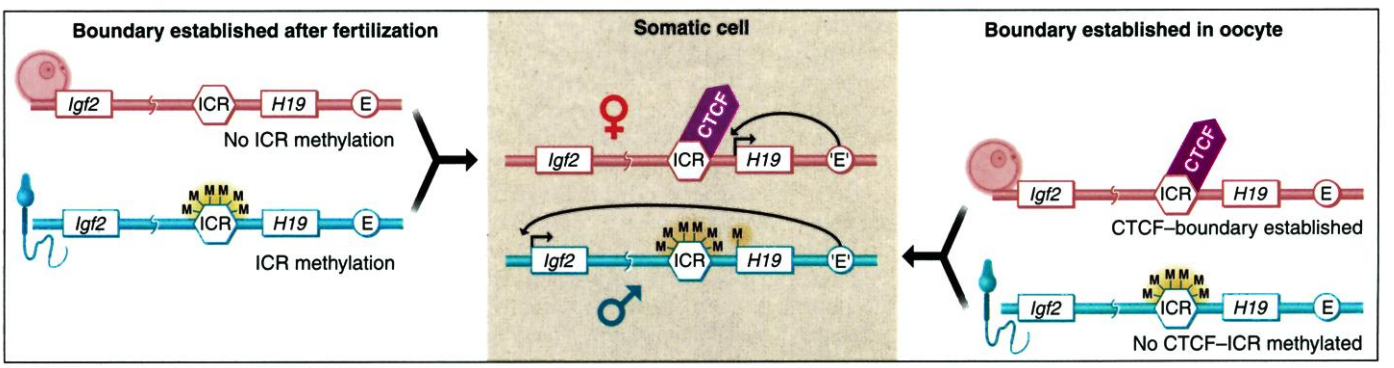
Two of the first reported and best studied imprinted genes are the *H19* and *Insulin-like growth factor II* (*Igf2*) genes. These genes are separated by 90 kb in mouse and are oppositely imprinted: that is, *H19* is expressed only from the maternal allele and *Igf2* only from the paternal allele. The proximity of the two genes and the fact that they are oppositely imprinted suggest that their imprinting is interdependent. It has been shown that these genes share enhancers (sequences that increase the activity of a gene's promoter) located downstream of the *H19* gene. The enhancers drive the expression of *H19* on the maternal chromosome and of *Igf2* on the paternal chromosome (*3*). But how is sharing of the enhancers regulated so that the appropriate parental allele is expressed on each chromosome? An early model (the enhancer competition model) suggested that *H19* and *Igf2* compete for the enhancers. According to this model, *H19* "wins" the competition on the maternal chromosome, possibly because of its more powerful promoter, and *Igf2* "wins" on the paternal chromosome because *H19* is unable to access the enhancers as it is heavily methylated.

However compelling the enhancer competition model might be, it still cannot explain the exclusive expression of *H19* on the maternal chromosome. An important clue that this model may not be valid comes from an experiment in which the enhancers were moved from their location downstream of the *H19* gene to a position between *H19* and *Igf2* (*4*). As a result, both alleles of *Igf2* were expressed, invalidating the hypothesis that *H19* is normally expressed on the maternal chromosome because of an inherently stronger promoter.

To explain these findings, Tilghman and colleagues proposed a chromatin boundary model of genomic imprinting. Chromatin boundary elements (insulators) were originally described in *Drosophila* as cis-acting elements that insulated a gene and its regulatory elements from position effects and that blocked transcription when placed between a gene and its enhancer (*5*). Tilghman has argued that an insulator located upstream of the *H19* gene isolates *Igf2* from its enhancers. When the enhancers are moved between the two genes (upstream of the putative insulator), the enhancers are accessible to *Igf2* but not to *H19*.

For insulators to operate in imprinted gene clusters, however, they must be regulated in an allele-specific manner; that is, they have to be inactivated on the repressed allele. Tilghman suggested that insulators could be inactivated by allele-specific methylation. An excellent candidate sequence for inactivation at the *H19*/*Igf2* locus is a 2-kb region located 2 kb upstream of the start of *H19* transcription. This sequence is methylated on the paternal allele (*6*) and has been alternatively called the imprinting control region (ICR), the differentially methylated domain, or the differentially methylated region. Deletion of 1.6 kb of this 2-kb region resulted in a loss of *H19* and *Igf2* imprinted gene expression. This demonstrates that the region is crucial for regulation of imprinted genes and indicates that the ICR could be acting as a methylation-sensitive insulator (*7*).

Now, the Tilghman and Felsenfeld reports in *Nature* (*1, 2*) demonstrate that the ICR does in fact act as an insulator. Using cell culture transfection assays, the two groups show that the ICR blocks transcription when placed between a gene and its enhancer. Tilghman and colleagues have gone one step further by proving that the ICR acts as an insulator in a mouse transgenic assay. Furthermore, the insulating ac-



Imprints in the sand. Imprinting (silencing of one allele) of the *H19* and *Igf2* genes in somatic cells (**center**) can be established in at least two ways. In the first model (**left**), the imprinting control region (ICR) is specifically methylated in male germ cells (sperm), and the ICR insulation region (boundary) is formed on the maternal allele after fertilization. In the second model (**right**), the ICR boundary is established in female germ cells (oocytes), and the ICR is methylated in sperm by default.

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tivity maps to two sets of maternal allele-specific hypersensitive sites at the ends of the ICR (*8*). Closer examination of these hypersensitive sites reveals that they overlap with several short CG-rich repetitive elements that are conserved between mouse, rat, and human (*9*). These repeats, which are the only similar upstream sequences between the human and mouse *H19* genes, are both necessary and sufficient for insulating activity in the cell culture assays (*1*). Intriguingly, the conserved zinc finger protein CTCF (CCCTC-binding factor) specifically binds to these repeats. Mutations that perturb insulator function abolish the binding of CTCF. When the cytosines in CG dinucleotides are methylated—suggesting (but not proving) that insulation is methylation-sensitive and may act exclusively on the maternal allele at the *H19/Igf2* locus—CTCF binding is also abrogated.

Recently, CTCF was shown to bind to the insulator region upstream of the chicken β-globin gene through a sequence that is similar to those of the *H19* repeats (*2*, *10*). Before its identification as an insulator binding protein, this ubiquitous DNA binding protein was described as both a transcriptional activator and a repressor (*11*). Thus, exactly what CTCF does is unclear. CTCF may serve as a loading station for additional factors required for insulation or, alternatively, CTCF may not be required at all. Additional experiments will be needed to test this scenario.

Although it is clear that the *H19/Igf2* ICR behaves as an insulator, could this boundary activity be a critical part of the imprinting mechanism? Could it be the mark that distinguishes the maternal and paternal alleles of imprinted genes? For genes to be imprinted, the imprint must first be established (usually in egg and sperm) and subsequently maintained. At least two models that incorporate the findings of the *Nature* papers can be envisioned to explain how the imprinting mark is acquired at the *H19/Igf2* locus (see the figure). In one model, the imprinting mark is established on the paternal allele: The ICR is methylated exclusively in the male germ line, and after fertilization the unmethylated maternal ICR forms an insulator between the gene and proteins such as CTCF. The alternative model predicts that the allelic mark is established as an insulator on the maternal allele during development of female germ cells. By default, the absence of CTCF on the paternal allele renders this allele a substrate for DNA methyltransferase (the enzyme that methylates CG repeats) in the paternal germ line. More experiments are required to determine if insulator activity and associated proteins are present in the germ line and during early embryogenesis. Furthermore, it remains to be proven in vivo that insulation is methylation sensitive, a necessary criterion if the insulator is to be considered the imprinting mark.

Do parental-specific insulator regions serve as regulatory elements for the expression of other imprinted genes? Although at least one other ICR has been identified in the imprinted gene cluster containing the *Snrpn* gene, the situation there is likely to be more complex because multiple paternally expressed genes are present on either side of the defined ICR. Furthermore, as other gene clusters have only recently been identified, it is too early to know whether the boundary model can be more generally applied.

The Tilghman and Felsenfeld studies unite the imprinting and insulator worlds of gene regulation. The mechanism by which insulator elements block enhancer-mediated transcription in vivo remains unclear. Doubtless, the *H19/Igf2* locus will continue to provide a fruitful model system to uncover the mechanism of these complex interactions.

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PERSPECTIVES: LIQUID CRYSTALS

New Banana Phases

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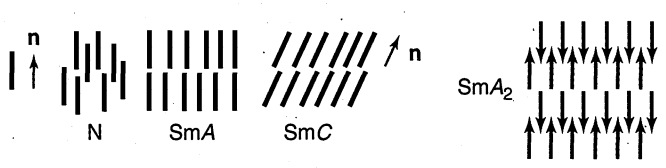
Liquid crystals (*1*) are materials that flow like a fluid but are optically anisotropic like a crystal. Because of their large responses to modest external disturbances, they are ideal components for flat-panel displays for computers and next-generation televisions. Two reports in this issue present exciting research on liquid crystals that are composed, at least in part, of V-shaped molecules referred to as bent-core molecules or "bananas" (*2*). On page 2181, Walba *et al.* (*3*) report a successful synthetic strategy for producing a ferroelectric phase from achiral bent-core molecules. And as Pratibha *et al.* report on page 2184 (*4*), new liquid crystalline phases form when bent-core molecules are mixed with a particular class of rodlike molecules.

There is a wide variety of liquid crystalline phases, most of which exhibit more symmetry than homogeneous, isotropic fluids but less symmetry than periodic crystals.



Common liquid crystal phases. (Left) Nematic (N), smectic-*A* (Sm*A*), and smectic-*C* (Sm*C*) liquid crystalline phases of rodlike molecules. **n** is a unit vector pointing along the direction of average molecular orientation. **(Right)** Bilayer smectic-A_2 (SmA_2) phase of polar molecules, with bilayer repeat unit and antiparallel alignment of electric dipoles.

The most common liquid crystalline phases are the nematic, smectic-*A*, and smectic-*C* phases (see left panel in the first figure). The bilayer smectic-A_2 phase (see right panel in the first figure) is relevant to the experiments of Pratibha *et al.* Many liquid crystal molecules are chiral; no rotation will superimpose their mirror images. The introduction of chirality of the same sign to some or all molecules has a profound effect on liquid crystalline structure: It converts a nematic into a cholesteric phase, in which the director **n** (see first figure) rotates in a helical fashion about an axis perpendicular to the plane of molecular alignment, and converts a smectic-*C* phase into a smectic-*C** phase, in which the projections of the molecular axes onto the smectic planes precess in a helical fashion about the layer normals.

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