

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

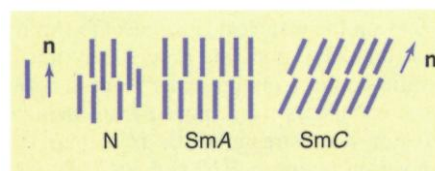
New Banana Phases

Tom C. Lubensky

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.

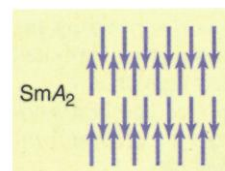
There is a wide variety of liquid crystalline phases, most of which exhibit more symmetry than homogeneous, isotropic fluids but less symmetry than periodic crystals.



Common liquid crystal phases. (Left) Nematic (N), smectic-A (SmA), and smectic-C (SmC) liquid crystalline phases of rodlike molecules. *n* is a unit vector pointing along the direction of average molecular orientation. (Right) Bilayer smectic-*A*₂ (SmA₂) phase of polar molecules, with bilayer repeat unit and antiparallel alignment of electric dipoles.

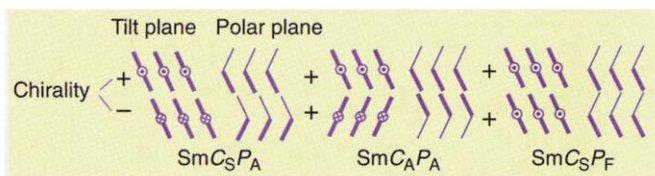
The most common liquid crystalline phases are the nematic, smectic-*A*, and smectic-*C* phases (see left panel in the first figure). The bilayer smectic-*A*₂ phase (see right panel in the first figure) is relevant to the experiments of Pratibha *et al.* Many liquid crystal molecules are chiral; no rotation 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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As first pointed out by Meyer *et al.* (5), the combination of chirality and molecular tilt of a smectic- C^* phase necessarily leads to a dipole moment in the plane of each layer perpendicular to the plane formed by



Bent-core molecules. (Top) Four views of bent-core molecules, showing the directions of the director $\mathbf{n}$ (along the bow of a bow and arrow) and the electric dipole moment $\mathbf{p}$ (along the arrow), pointing, respectively, to the left, out of the paper, and into the paper. Each molecule has two arms lying in the $\mathbf{n}$ - $\mathbf{p}$ plane with a relative orientation of order 120° . Counterclockwise rotation about $\mathbf{p}$ produces the four views on the right. In the first two views on the right, the thick arm has rotated out of the paper and the thin arm into the paper. These configurations have positive chirality if the molecules are aligned in a plane. Clockwise rotation produces configurations of negative chirality. (Bottom) Some smectic phases of bent-core molecules. Molecules in each layer align with common directions of $\mathbf{n}$ (tilted relative to the layer normal) and $\mathbf{p}$, and each layer has either positive or negative chirality. The nomenclature is explained in the text.

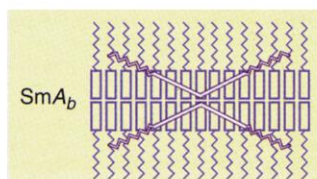
the local layer normal and the molecular orientation axis. A smectic- C^* phase is therefore a ferroelectric, with an in-plane polarization that responds easily to external electric fields or boundary conditions. Its switching properties are appropriate for display applications.

The synthesis of bent-core molecules, consisting of V-shaped aromatic rigid cores attached to alkyl tails, opens the door to a rich array of new phases not found in rodlike molecules. Seven distinct phases, tentatively labeled B_1 to B_7 (where "B" stands for "banana"), of bent-core molecules have been identified (6). However, not all of them have been fully characterized, and there is as yet no universally accepted nomenclature for these phases and their derivatives. It is known that the B_2 and B_7 phases are smectic phases, consisting of stacks of fluid layers with some internal orientational order.

Building on earlier work by Link *et al.* (7), Walba *et al.* enumerate possible structures for bent-core smectic phases and propose a nomenclature (3). Because of packing constraints, bent-core molecules tend

to segregate into planes, with their polar axes aligned along a common direction in a given plane. In all smectic bent-core phases so far characterized, the molecules rotate about their polar axes to produce a director tilt, like that of a smectic- C phase, relative to layer normals. Because of the common polar alignment, this director tilt creates a chiral configuration, whose sign depends on the direction of rotation about the polar axis (see top panel in the second figure). Smectic bent-core phases can therefore be classified by specifying the relative tilt and relative polar directions in neighboring layers. Walba *et al.* introduce the specification $\text{SmC}_X P_Y$, where SmC indicates a tilted structure and P a polar structure. The subscript X may be S for synclitic (same direction) tilt or A for anticlinic (opposite direction) tilt of neighboring

layers, and the subscript Y is F in case of ferroelectric or A in case of antiferroelectric alignment of dipoles in neighboring layers. All known properties of the B_2 phase are consistent with the $\text{SmC}_S P_A$ phase; those of the B_7 phase are consistent with the $\text{SmC}_A P_A$ phase (see bottom panel in the second figure). The $\text{SmC}_A P_A$ phase breaks achiral symmetry because all of its layers have the same chirality. The constituent molecules are not chiral, and re-



molecules (red) dissolve in the aromatic smectic region with as many polar directions up as down. The bows align along a common direction to produce in-plane biaxial order.

gions of opposite chirality are therefore expected to coexist, as is observed.

Walba *et al.* observe that when projected onto the polar plane containing the director and the layer normal, the arms of the bent-core molecules in antiferroelectric phases are synclitic, whereas those in ferroelectric phases are anticlinic across the interface between two layers. They

synthesized a bent-core molecule with arms that prefer anticlinic alignment in the polar plane and produced the first ferroelectric smectic phase, $\text{SmC}_S P_F$, composed of achiral molecules. This phase spontaneously breaks achiral symmetry and, as in $\text{SmC}_A P_A$, exhibits coexisting regions of opposite chirality.

Pratibha *et al.* describe a successful strategy for mixing bent-core and rodlike molecules (4). They have synthesized new bent-core and biphilic rodlike molecules. The latter consist of aromatic heads attached to single alkyl chains, which are matched to the aromatic centers and alkyl tails of the bent-core molecules. The rodlike molecules form a bilayer smectic phase with alternating aromatic and alkyl regions, into which the bent-core molecules can easily dissolve. At concentrations of bent-core molecules between 4 and 13 mole percent, a new biaxial bilayer smectic- A_b phase is observed (see the third figure). In this phase, the aromatic cores of the bent-core molecules are confined to the aromatic regions of the smectic bilayers while their alkyl tails penetrate the smectic alkyl regions. As many bent-core dipoles point up as down, the molecular bows align along the common in-plane direction $\mathbf{n}$. The smectic- A_b phase is invariant under $\mathbf{n} \rightarrow -\mathbf{n}$. Thus, unlike the smectic- C phase, it is biaxial rather than uniaxial in the smectic planes, as verified by the observation of half-integer orientational defects not permitted in in-plane uniaxial systems.

Many advances in liquid-crystal science over the past 30 years have resulted from the directed synthesis of new molecules that form new phases. The reports (3, 4) continue this trend by providing new molecular design paradigms for controlling the tilt of bent-core molecules in neighboring layers and for mixing together molecules of different shapes. These paradigms offer new tools to control microscopic and macroscopic structure and response to external fields. They are sure to find uses in the future, for example, to create new ferroelectric switches or biaxial phases of bilayer membranes composed of amphiphilic molecules designed to dissolve bent-core molecules.

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