molecules or atoms but rather by motions of electrons. This effect is much weaker than LC molecular switching and typically requires much larger devices for the same degree of interaction with light at a given modulating field strength, but it can deliver blistering speed, an acceptable tradeoff for ultrafast single-channel modulators. In order to achieve useful NLO-based switching, however, supermolecular stereocontrol is again required, one in which very specific arrays of functional groups are oriented correctly in space. Because such materials are typically colored, these functional arrays are often termed NLO chromophores.

The most straightforward approach to organic NLO materials involves orientation of NLO chromophores along the polar axis of a sample with macroscopic polar symmetry. A very intensively studied approach for achieving this structure involves application of an external electric field to an organic polymer film containing NLO dye molecules with large dipole moments, using an external electric field, then freezing in the resulting polar structure by lowering the temperature of the sample to below the polymer glass transition. Great success in obtaining films with useful EO properties has been achieved in this way, and ultrafast optical circuits using such poled polymers are being demonstrated. However, problems remain, and several alternative approaches are under investigation.

Because FLCs spontaneously self-assemble into a polar structure, and techniques for processing FLC films on VLSI silicon are already relatively highly developed, FLC materials with useful NLO properties would complement poled polymers, providing increased flexibility in design and fabrication of NLO switches. Early NLO experiments with FLCs, however, showed that typical materials used in displays have NLO figures of merit on the order of 10^5 too small. Some understanding of the molecular origins of the polar structure occurring in FLCs has allowed the design of materials improved by about 10^4 in EO properties (*7*); however, at least an additional order of magnitude improvement is required in order to create useful ultrafast FLC NLO chips.

Until recently, this goal seemed elusive because of a very fundamental LC principle: LC molecules possess a large steric aspect ratio (that is, the molecules are long and skinny). Furthermore, in the FLC phase the long axis of the molecules is perpendicular to the polar axis of the material. Because most NLO chromophores are long functional arrays, they tend to orient perpendicular to the FLC polar axis as well, leading to incorrect supermolecular stereochemistry and poor NLO properties.

This problem now appears tractable, given the recent synthesis and characterization in our laboratories of FLC side-by-side dimer structures wherein the chromophore of the prototype NLO dye disperse red 1 (DR1) orients along the polar axis (normal to the LC long axis) in the FLC phase (*10*). The materials possess excellent liquid crystallinity; polarized light spectroscopy, ferroelectric polarization measurements, and powder x-ray scattering combine to show that the materials self-assemble to give the proper supermolecular stereochemistry for NLO. Thus, it seems reasonable to hope that FLCs with NLO properties on a par with poled polymers will be achievable, paving the way for the creation of ultrafast VLSI-FLC EO modulators similar in basic structure to the device shown in the figure.

Several key issues need addressing before realization of this goal, however. These issues revolve around the fact that it will likely be necessary for the light to propagate parallel to the plane of the FLC film in NLO-based devices, requiring guided wave optics. Very little work on FLC wave guiding has been accomplished to date, and key aspects of the problem such as temperature sensitivity and optical clarity have not yet been addressed. However, it is clear that because their unique supermolecular structure, FLCs offer a highly attractive potential approach to both highly parallel and ultrafast transmission of information with light.

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A Heterodimeric Transcriptional Repressor Becomes Crystal Clear

Brenda J. Andrews and Michael S. Donoviel

Over the past decade or so, studies of cell-type determination in budding yeast have consistently affirmed the predictive power of genetics—complex hypotheses, based solely on the analysis of mutants, have largely withstood the scrutiny of biochemists and molecular biologists. Now, the crystal structure of a1/α2 repressor, solved by Li *et al.* and described in this issue (*1*), provides a decisive molecular view of interactions suggested by genetic functional studies in vivo. In addition, the structure provides a first glimpse at what is likely to be a common mechanism by which the activity of transcriptional regulatory proteins is altered by interaction with different partners.

Saccharomyces cerevisiae exists as three cell types with distinct physiological properties and specialized roles during the yeast life cycle (*2*). Haploid cell type is determined by a single genetic locus, *MAT*, which encodes master regulatory proteins that dictate the expression of sets of cell type–specific genes. Haploid **a** cells carry the *MAT***a** allele and express a set of **a**-specific genes. In the other haploid cell type, α, which carries the *MAT*α allele, **a**-specific genes are turned off while another set of genes (α-specific genes) is turned on. Haploid **a** and α cells can mate to form the third specialized cell type, the **a**/α diploid cell.

Transcriptional repression is crucial in controlling the expression of cell type–specific gene sets (*3*, *4*). First, in both α and **a**/α cells, the MAT α2 gene product α2 collaborates with the ubiquitous transcription factor Mcm1 to form a heterodimeric repressor which binds to the **a**-specific gene operator that lies upstream of **a**-specific genes. As a result of α2-Mcm1 binding, **a**-specific genes are turned off (*5*, *6*). Second, in **a**/α diploid cells, α2 performs another function; it binds cooperatively with the *MAT***a** gene product **a**1 to the haploid-specific gene operator to turn off haploid-specific genes (*6*, *7*). The **a**1 and α2 proteins are related: They belong to the homeodomain family of proteins that regulate transcription in a wide range of eukaryotic organisms (*8*). Like some other homeodomain

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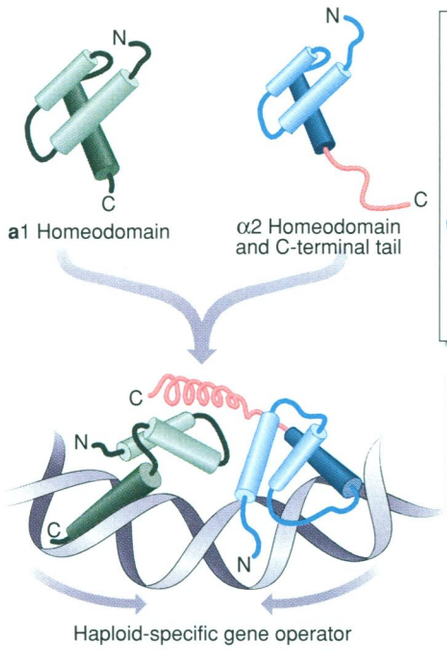

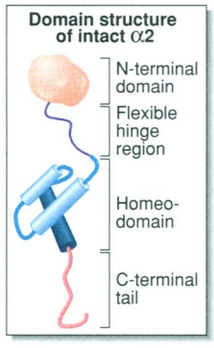

ILLUSTRATION: K. SUTLIFF

The yeast a1/α2 repressor bound to DNA. In the **a**1/α2 heterodimer, the carboxyl terminal (C-terminal) tail of α2 (pink) forms an amphipathic helix that contacts the homeodomain of **a**1. Binding of **a**1/α2 induces a bend in the DNA that is important for correct repressor-DNA interaction. Cylinders, helical regions.

proteins, the specificity and affinity with which α2 binds to DNA are enhanced by interaction with a partner protein. In particular, association with Mcm1 or **a**1 endows α2 with the appropriate binding specificity to repress two distinct classes of target genes. On its own, the **a**1 homeodomain protein cannot bind DNA.

We know how α2 interacts with DNA (*9*). As in other homeodomain proteins, the homeodomain of α2 folds into three helices, the most carboxyl-terminal of which inserts into the major groove of DNA (see figure; *8-10*). Genetic experiments have defined two regions of α2, adjacent to the homeodomain, that specify formation of a complex with different partners. Mutations in the so-called "flexible hinge" region of α2, located amino terminal to the homeodomain, diminish the cooperative interaction with Mcm1 and reduce the repression of **a**-specific genes (*11*). In contrast, mutations in a "tail" domain, which lies carboxyl terminal to the homeodomain, specifically impair both the cooperative interaction with **a**1 and the repression of haploid-specific genes (*12*). These hinge and tail regions are sufficient to confer dimerization specificity: A heterologous homeodomain can be directed to interact with Mcm1 simply by adding the flexible hinge and will interact with **a**1 if fused to the carboxyl terminal tail. An important piece of structural information adds to the picture: When α2 alone is bound to DNA, or is uncomplexed in solution, the carboxyl terminal tail is disordered. However, solution nuclear magnetic resonance studies show that the tail assumes a helical structure when it is bound to **a**1 (*9*, *10*, *13*). Together, these observations implicate the carboxyl terminal tail of α2 as the key interface with the **a**1 protein in the **a**1/α2 repressor complex.

The molecular picture of an active **a**1/α2 repressor complex impressively confirms this prediction. Li *et al.* have solved the structure of a ternary complex containing an **a**1/α2 heterodimer bound to a 21–base pair operator sequence. The carboxyl terminal tail of α2 indeed provides the sole contacts with **a**1 in the heterodimeric complex (*1*). A portion of the tail forms a short amphipathic helix with three leucine residues which project from the surface of the helix into a hydrophobic pocket on the **a**1 homeodomain. Mutation of these leucine residues, or another hydrophobic residue that stabilizes the tail helix, diminishes the ability of **a**1 and α2 to repress transcription in yeast. The precise correlation between an active repressor in vivo and a helical tail indicates that interactions of the carboxyl terminal tail of α2 and the homeodomain of **a**1 are crucial for creating a functional repressor complex.

How is the DNA binding activity of the **a**1/α2 complex affected by the heterodimer interface? The structure of the **a**1/α2–DNA ternary complex rules out the simple model that the contacts made between the α2 homeodomain and DNA are altered as a result of heterodimerization. Li *et al.* (*1*) found that contacts between the α2 homeodomain and DNA in the context of the heterodimer are virtually indistinguishable from those seen in the structure of an α2 monomer bound to DNA. Two observations make this finding somewhat surprising. First, although α2 alone binds to unbent DNA, binding of the **a**1/α2 heterodimer causes a pronounced bend in the DNA, making additional protein-DNA contacts possible (*9*, *14*). Second, a version of α2 that is mutated in three key residues that contact the major groove of DNA binds DNA poorly on its own but still functions well in the **a**1/α2 heterodimer (*15*).

How then is the binding specificity of the **a**1/α2 heterodimer achieved? When interpreted in the context of in vivo studies, the structure of the ternary complex suggests that the increased DNA binding specificity of the heterodimer has several roots. First, the **a**1 homeodomain makes significant contacts with the DNA—the heterodimeric complex will bind only to sites that provide contacts required by both partners. Perhaps the **a**1-specific DNA contacts can compensate for defects in α2 binding, accounting for the relative resistance of the heterodimer to mutation of residues in α2 that contact DNA. If this were true, the DNA binding mutant of α2 protein might be exquisitely sensitive to mutation of key residues in **a**1 that contact the DNA. Second, noncontacted bases in the DNA sequence may also add to the stability of the complex because an **a**1/α2 operator sequence must accommodate a significant bend in order to form a productive repressor complex. Finally, interactions involving the carboxyl terminal tail of α2 and the homeodomain of **a**1 are clearly crucial for the specificity of repressor binding. As discussed above, the protein-protein contacts between **a**1 and α2 do not appear to alter notably their DNA binding helices. Rather, the heterodimer interface may increase specificity by dictating the precise spacing of the DNA binding helices of the two partners. In this regard, it is of particular interest that small alterations in spacing between the **a**1 and α2 binding sites within the operator sequence severely reduce both heterodimer binding in vitro and repression in vivo (*16*). Jin *et al.*, in experiments also described in this issue, extend this observation (*17*). They designed altered α2 proteins with small insertions into the tail of α2 between the homeodomain and the helix that contacts **a**1. These α2 insertion mutants allowed efficient repression of transcription through both wild-type **a**1/α2 sites as well as operator sequences with increased spacing between the half-sites. Increased flexibility at the heterodimer interface may allow increased flexibility in the binding site.

The structure of the **a**1/α2–DNA complex provides a detailed molecular view of the pairing of α2 with one of its partners. This pairing is clearly central to an elegant mechanism for the combinatorial control of gene expression. Yeast and other eukaryotic cells use highly related proteins to mediate combinatorial control. Li *et al.* (*1*) describe a number of other homeodomain proteins which may interact with partners by a flexible tail analogous to that found next to the α2 homeodomain. For example, interaction of the *Drosophila* extradenticle homeodomain protein with other homeodomain regulators requires short regions next to the homeodomain (*18*). Future structural studies may well reveal a highly conserved means of using heterodimerization to allow a small number of proteins to influence many regulatory decisions.

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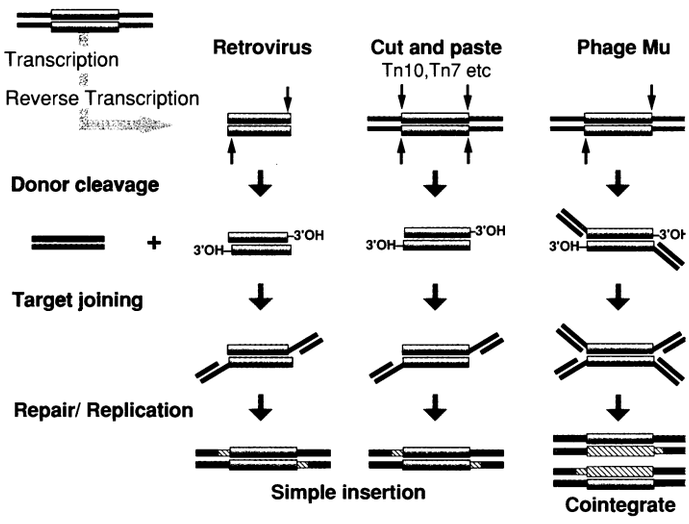
Unity in Transposition Reactions

Nancy L. Craig

The process of transposition, in which pieces of DNA move around the genome, was made famous by Barbara McClintock's discovery of "jumping genes" and the surprising plasticity of DNA (*1*). Other biological transactions also make use of this process: the acquisition of bacterial genes for antibiotic resistance, the replication of certain bacteriophages, the integration of retroviruses, and the intracellular movement of retroviral-like elements. New evidence points to unexpected parallels among these many transposition events: They all occur by similar DNA breakage and joining reactions. Moreover, the structures of transposition proteins from very different biological sources have remarkable overall structural similarity, even though they lack extensive primary sequence homology.

Central to all transposition reactions are breakage events that precisely expose the 3′ tips of the transposable element; these exposed 3′ tips are then joined to the target DNA (*2*). Sometimes DNA cleavage reactions also occur at the 5′ tips of the element; whether this occurs has a profound influence on the structure of the transposition products. Thus, although all transposition reactions involve DNA breakage and joining, several different types of recombination products can emerge, depending on which DNA strands are broken and joined (see figure).

Retroviral integration (*1*–*3*) and the replication of phage Mu (*1*, *2*, *4*) exhibit breakage and joining at only the 3′ ends of mobile elements. In retroviral integration, the viral RNA genome is converted to double-stranded DNA by reverse transcription. This DNA product is then cleaved by the retrovirally encoded integrase to expose 3′-OH ends at the embedded tips of the actual retroviral DNA. Strand transfer reactions then join these exposed 3′ ends to staggered positions on the target DNA, one transposon end joining to one target strand and the other end joining to a displaced position on the other target strand. As a result, the transposon is covalently joined to the target DNA but is flanked by short gaps that reflect the staggered positions of target joining; host DNA repair functions then repair these flanking gaps. The resulting product, in which the retroviral DNA is covalently joined to the target DNA, is called a simple insertion (see figure).



The DNA-processing reactions that underlie the translocation of three mobile elements. Gray boxes, mobile elements; blue lines, flanking donor DNA; black arrow, cleavage at the 3′-OH ends; green arrow, cleavage at the 5′ ends; red line, target DNA; cross-hatching, DNA replication.

In the case of bacteriophage Mu replication, the phage DNA is embedded in host chromosomal DNA. DNA cleavage reactions executed by MuA protein, the Mu-encoded transposase, introduce single-strand nicks at both tips of the element. These cleavages cleanly expose the 3′-OH ends of the transposon, separating them from flanking bacterial DNA but leaving the transposon covalently linked at its uncleaved 5′ ends to flanking DNA; the exposed 3′ ends of the transposon are then joined by strand transfer reactions to staggered positions on the target DNA. This transposition product is then replicated by host DNA replication to generate a product called a cointegrate in which the donor backbone, target, and two transposon copies are linked (see figure). In some cases, such as Tn3, another element-encoded recombination system further processes the cointegrate to generate a target molecule containing a simple insertion and regenerate the donor (*5*).

Identical chemical steps executed by element-encoded transposition proteins underlie retroviral integration and Mu replication: DNA cleavage reactions expose the 3′-OH ends of the elements, followed by strand transfer reactions that covalently join these 3′ element ends to the target DNA. Although the products of retroviral integration and Mu transposition are distinct, their differences arise not from the transposition reactions per se but rather from the state of the DNA substrate, in particular at the 5′ ends. Mu remains linked to the donor site while also inserting into the target site, because no cleavage occurs at the 5′ ends of the element; by contrast, in the retrovirus the substrate DNA contains only the transposable DNA segment.

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