within the middle of the sequence (*7–9*). The ternary complex described by Geiger *et al.* includes a yeast variant of TFIIA that consists of the full-length small subunit and a large subunit with an internal deletion, resulting in a functional but truncated molecule (*10*). The two chains of TFIIA embrace to form a structure consisting of a six-stranded β-barrel and a four-helix bundle, in which half of each domain is contributed by each of the two subunits. In the absence of either the large or the small subunits, a stable globular protein is unlikely to form. Such a fold is not typical of the modular organization of most transcription factors and should alert us to the dangers of using deletion mutants to map interaction domains in the absence of structural information.

Most revealing is that only relatively small portions of TBP's exposed surface in the binary complex become buried upon formation of the ternary complex. TBP also has restricted contact surfaces in the TFIIB triple complex (*4*) and in its interactions with other basal factors (*11*). These observations suggest that factors may enter the preinititation complex by contacting rather limited regions of different subunits within the complex, relying upon the relative spatial disposition of these elements to obtain specificity. In the TFIIA ternary complex, these interactions involve specific contacts to limited regions of TBP and nonspecific interactions with the phosphate backbone of the DNA. This mode of recognition may partly explain how TBP and TFIIA can participate in multiple interactions with other components of the transcriptional machinery. Indeed, isolation of TBP-containing complexes from *Drosophila* and human cells suggest that this essential transcription factor is in a stable complex with numerous tightly associated subunits, termed TAFs, that together form TFIID. Some of the TAFs bind both TFIIA and TFIIB. Thus, the formation of an active preinitiation complex likely requires multiple contacts between TBP, TFIIA, TFIIB, and TAFs.

It is not known at present whether the promoter/preinitiation complex can form via alternative contacts between its constituents, depending upon the core promoter sequences and adjacent DNA-binding proteins. We also do not know how the TAFs interact with the TBP/DNA/TFIIA structure reported here because some of the subunits of TFIID directly contact TFIIA (*7*). As more structural information becomes available, it will become clear how other components of the basal transcription apparatus—such as TFIIE, TFIIF, and the subunits of RNA polymerase II—fit into the framework revealed by the TFIIA and TFIIB complexes and how TFIIA may transduce activation signals from enhancer-bound regulators.

It is hard to believe that only 6 years ago we had just obtained the first few components of the preinitiation complex as purified proteins that could restore accurate initiation of transcription in vitro. Now that high-resolution structures of partial initiation complexes are accumulating rapidly, we may soon have a detailed picture of the entire transcription machinery and will begin to understand how this amazing complex controls the expression of some 100,000 genes in the eukaryotic cell.

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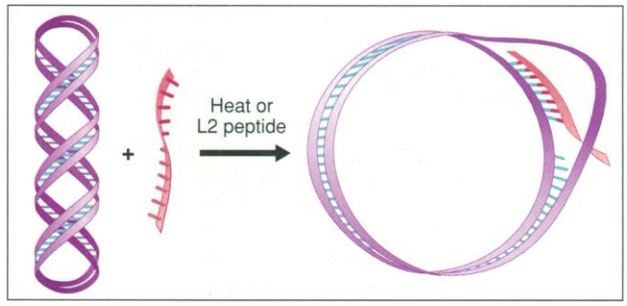
Getting Down to the Core of Homologous Recombination

Andrzej Stasiak

Can a small piece of a big enzyme perform a reaction normally catalyzed by the whole protein? It seems that the answer is yes, at least when the piece is a 20–amino acid peptide derived from RecA, a bacterial protein that repairs and recombines DNA, and when the reaction is a certain kind of DNA strand exchange. In this issue, Voloshin and coworkers (*1*) present experiments that illuminate a crucial step in homologous recombination, the process by which DNA molecules with similar genetic information line up side by side and exchange strands. Such reassortment of the genetic material creates new genetic traits in the offspring and thus drives the process of evolution.



Strand exchange between supercoiled double-stranded DNA and single-stranded DNA. The supercoiled molecule becomes relaxed as strand exchange progresses. This process of relaxation drives forward the reaction in which every opening of base pairs in the substrate duplex is compensated by formation of new base pairs in the heteroduplex region. The reaction stops when the relaxation of supercoiled DNA is attained (*4*). In this schematic drawing the primary helicity of DNA is not shown. For every 10 base pairs exchanged (one turn of DNA helix), one superhelical turn is relaxed.

In their search for the regions of the RecA protein that can mediate DNA strand exchange, these authors turned to the crystal structure of RecA, solved earlier (*2*). Although there is no DNA in these crystals, RecA monomers form helical structures that are similar in shape to RecA-DNA filaments visible under the electron microscope when RecA binds to DNA. Crystallized RecA monomers have two disordered loops, as if they were lacking stabilizing contacts with DNA. Comparison of the crystal structure of RecA with the RecA-DNA filaments (*3*) suggests that these disordered loops (L1 and L2) are in a good location to contact the DNA. Therefore, Voloshin and co-workers synthesized short peptides equivalent to individual disordered loops and assayed the activities of these peptides. One of the 20–amino acid peptides, corresponding to loop 2, was able to bind to single- and double-stranded DNA. In addition, while binding to single-stranded DNA, this peptide decreased stacking of nitrogenous bases, the building blocks of DNA, mimicking RecA's ability to stretch and partially unstack single-stranded DNA. This result is hardly surprising; after all, DNA binding domains are expected to bind to DNA, although they may show a lower affinity when taken out of the context of the whole protein.

However, the authors went further and challenged the peptide with the seemingly impossible task of catalyzing DNA strand

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exchange. They chose a particular type of DNA strand exchange reaction, namely, that between supercoiled duplex DNA and single-stranded DNA complementary to one of the strands in the duplex. This reaction has a unique advantage: It is driven forward by the free energy of DNA supercoiling (see the figure). Indeed, at elevated temperatures (but still below the melting temperature of DNA), these reactions can proceed spontaneously without participation of any proteins (*4*). This temperature elevation is necessary for the initiation of spontaneous strand-exchange reactions, suggesting that premelting changes in the single-stranded DNA or supercoiled duplex DNA are necessary for the homologous pairing.

RecA protein can promote pairing between these DNA molecules at temperatures well below the threshold point of the spontaneous reactions. RecA likely induces in DNA a premelting state that is necessary for homologous recognition between DNA molecules. This state probably involves a partial unstacking of DNA bases. So it follows that the 20–amino acid peptide with DNA-unstacking activity could substitute for the entire RecA molecule and catalyze the reaction, although it is less efficient than RecA (*1*).

If a small peptide can mediate DNA strand exchange, what is the function of the remaining 332 amino acids of RecA? Because the reaction used by Voloshin *et al.* was energetically driven by the relaxation of torsional stress in supercoiled DNA, the peptide was not required to do too much; it needed only to initiate the strand exchange, which would then proceed further spontaneously. In natural recombination reactions in cells, DNA supercoiling will rarely drive the reaction in this way. Usually, DNA strand exchange is energetically costly—long DNA molecules have to be rotated, proteins on DNA must be removed, and the strand-exchange reaction has to pass through heterologous regions that are not well paired. Whole RecA protein hydrolyzes adenosine 5′-triphosphate (ATP), and the energy so harnessed drives the strand exchange reaction (*5*). The protein domains that convert chemical energy into a directed motion are certainly bigger than 20 amino acids. There are also probably many other domains that are needed for the full activity of RecA—for example, domains that contact LexA repressor during proteolytic cleavage, a reaction necessary for the response of the cell to DNA damage (*6*). Thus, in real life RecA needs all of its 352 amino acids.

However, the part of RecA that helps two homologous DNA molecules to recognize each other is very likely the L2 loop, or a portion of it. Its ability to unstack DNA bases would allow RecA to stretch and unwind the DNA helix. A yeast recombination protein, Rad51, and its human analog also stretch and unwind DNA (*7*); these additional examples of a stretched DNA structure facilitating homologous recognition of identical sequences point to a universal mechanism of homologous recognition. When protein-free DNA is stretched by an external force, there is a transition from the classical Watson-Crick structure to the stretched form, with a roughly 50% extension of the length (*8*). This increase corresponds to the DNA extension in filamentous RecA-DNA complexes (*9*). Perhaps RecA, by binding to DNA, uses its L2 region to induce a structural transition in DNA from a regular helix to the stretched configuration.

Until now, experiments with the whole RecA protein could not reveal the mechanism by which RecA stretches and unwinds DNA. Now that Voloshin *et al.* have identified the active core peptide of RecA that can cause stretching and unwinding, the field of DNA recombination should soon know exactly how two homologous DNA molecules recognize each other before they exchange their genetic material.

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Dengue Hemorrhagic Fever

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An alarming emerging disease is caused by dengue viruses, which have escaped their original home in Asia to spread to the tropical Americas. Dengue infection, usually resulting in flu-like symptoms, now sometimes causes a much more dangerous illness—dengue hemorrhagic fever, also known as dengue shock syndrome—which can be fatal in infants and young children (*1*). Thus, the new results of Olson *et al.* in this issue of *Science* (*2*) are particularly welcome; they give hope that we may be able to control dengue fever by genetically altering the organism that transmits the disease, the mosquito.

Most research on dengue virus has focused on vaccine development and on better methods of eradicating mosquitoes. But a more promising approach may be to engineer mosquitoes so that they can no longer transmit disease. If mosquitoes that have been engineered to be resistant to the virus are released into the natural population, they should decrease the transmission of the disease. To accomplish this, a three-pronged approach is necessary: We must (i) be able to genetically engineer mosquitoes in the laboratory, (ii) know how to move the genes into mosquito populations in the wild, and (iii) understand the population genetics and transmission properties of the target mosquitoes.

The results of Olson *et al.* are an important step in successfully constructing mosquitoes that cannot transmit disease: their results provide a proof-of-principle that we can really block dengue transmission by genetic engineering. Olson and co-workers (*2*) have used a Sindbis virus to express an antisense RNA derived from the dengue viral genome in the mosquito *Aedes aegypti*. The presence of this antisense RNA in the mosquito prevents dengue infection of the salivary glands and halts subsequent transmission of the virus.

This strategy of creating "intracellular immunity" is the first successful effort to express an exogenous gene that confers resistance to an important human pathogen. Nevertheless, more needs to be done. The new Sindbis viral constructs are not permanently integrated into the mosquito genome, so the resistance to dengue virus cannot yet be passed down to new generations of mosquitoes. But when this hurdle is overcome, these dengue-resistant mosquitoes can be tested in field trials for effectiveness in real-world situations.

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