PERSPECTIVE

Visualizing Mu transposition: assembling the puzzle pieces

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Mobile genetic elements, once a curiosity of maize genetics, are now known to be remarkably prevalent in the genomes of organisms ranging from pathogenic bacteria to humans. The varied consequences of these elements include the alteration of gene expression and the spread of drug resistance genes among bacteria. Additionally, many viruses and bacteriophages that covalently integrate their own genome into their hosts' do so by mechanisms similar to those used by more domesticated mobile elements that do not normally leave the cell (for review, see Curcio and Derbyshire 2003).

Bacteriophage Mu was the first transposition system studied *in vitro*, and has in many ways served as a paradigm for understanding the mechanisms of mobile DNA elements (Chaconas and Harshey 2002). In this issue of *Genes & Development*, Chaconas, Ottensmeyer, and colleagues (Yuan et al. 2005) present an electron microscopy-based 3D model of the protein–DNA complex responsible for Mu transposition (the transpososome). This model not only ties together years of accumulated biochemical and structural data, but also provides interesting new insights.

Mu is a member of the first characterized and perhaps most well studied family of mobile elements, often referred to as the “classical DNA transposons” or the “DDE transposons.” The latter name reflects the conserved active sites of the element-encoded transposase enzymes, which contain three carboxylate side chains that bind catalytic Mg⁺⁺ ions (Mizuuchi and Baker 2002). This large family includes the bacterial transposons Tn5, Tn7, and Tn10 as well as phage Mu, which is covalently inserted into the host genome and, in lytic phase, replicates via a massive burst of transposition. The family can also be extended to include retroviruses such as HIV, whose integration is catalyzed by an enzyme closely related to the DDE transposases.

A generalized pathway for the transposition of these elements is shown in Figure 1. The first step is simple hydrolysis of the DNA backbone at the 3' ends of the element. This is reflected in the structural similarity between the active site-containing domains of these trans-

posases and that of RNase H, which hydrolyzes the RNA strand of RNA–DNA hybrids (Rice and Baker 2001). However, unlike nucleases that use only water as a nucleophile, transposases can also use the 3' hydroxyls released in the first step to attack another DNA segment (the “target”). This strand transfer reaction is rather odd in that there is no obvious chemical driving force (the total number of phosphodiester bonds is unchanged). It appears to be driven forward solely by product binding energy. As a consequence these “enzymes” do not usually turn over, and in the Mu case the final complex is known to be so stable that it blocks replication until it is removed in an ATP-dependent fashion by ClpX (Nakai et al. 2001).

The fate of the second DNA strand of the original duplex varies. In replicative transposition systems such as Mu it remains intact, and the resulting branched DNA is later converted to a replication fork. In Tn7 transposition, it is cleaved by a second nuclease (Sarnovsky et al. 1996), whereas in Tn10 and Tn5 transposition it is cleaved by successive hairpinning and hydrolysis reactions catalyzed by the same active site as the one that mediates the initial cleavage (Kennedy et al. 1998; Bhasin et al. 1999). The latter pathway bears striking resemblance to the series of reactions carried out by the RAG proteins during immunoglobulin gene assembly in higher organisms (Jones and Gellert 2004).

Transposition takes place within a large protein–DNA complex (termed a transpososome) that often includes additional regulatory features. For instance, it is important to the health of the transposon that the catalytic events at its two ends be coordinated. Biochemical experiments on Mu followed by structural studies of Tn5 transposase showed that this can be enforced by catalysis *in trans* (Aldaz et al. 1996; Savilahti and Mizuuchi 1996; Davies et al. 2000). Tight, specific binding of the transposase to the sequences near the transposon ends is accomplished not by the active-site domain itself but by one or more additional DNA-binding domains. Within the transpososome, the protomer that is bound specifically to one transposon end catalyzes the chemical events at the other end, and vice versa.

Mu is a system particularly rich in regulatory features: For example, transpososome assembly is stimulated by an enhancer sequence found within the Mu genome that is bound by a separate domain of the transposase (MuA

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Figure 1. General pathway for transposition of bacteriophage Mu and related mobile elements. An oligomer of transposase protein (blue) bridges the ends of the element (bold lines, not to scale). Nicking at the host–element junction produces free 3' hydroxyls that then attack the phosphodiester backbones of a target DNA (red) in a transesterification reaction.

protein), while yet another transposase domain interacts with MuB protein, which mediates Mu's choice of distal DNA rather than its own genome as a target (Chaconas et al. 1996). While some simpler systems require only a dimer of transposase, a minimal functional Mu transpososome contains two 50-bp DNA segments, each bound by two MuA protomers. The role of the additional protomers is not entirely clear, but they may participate in regulatory functions.

Biochemical understanding is often greatly illuminated, if not driven, by structure. The structure of Tn5 transposase in complex with DNA has been an enormous boon to the field (Steiniger-White et al. 2004). However, it is a smaller, simpler system than Mu, and given the wide variety seen in transposition systems, extrapolating generalities from one structure would be the biochemical equivalent of deriving a curve from a single point. Structural studies of Mu transpososomes have been stalled by a problem all too familiar to those interested in large multicomponent complexes: Although the structures of nearly all the individual domains of MuA have been determined by NMR or X-ray methods, the entire complex is too large for NMR and while not utterly recalcitrant to crystallization, diffracts extremely poorly (P. Rice, unpubl.). The researcher is thus left with a tantalizing collection of puzzle pieces (Fig. 2).

Chaconas, Ottensmeyer, and colleagues (Yuan et al. 2005) have now used scanning transmission electron microscopy to produce a 3D reconstruction of the Mu transpososome that helps break this barrier. The new picture is even in "color": By also using electron spectroscopic imaging, they have specifically determined which parts of the mass correspond to DNA rather than protein. Although the resolution of the reconstruction is relatively low (34 Å), by combining it with a higher-resolution MuA monomer (16 Å) and with atomic domain structures and extensive biochemical data, the authors were able to create a detailed model of the entire complex.

The new Mu transpososome model resembles a large V. Each side of the V consists of two protomers of MuA with a DNA segment snaking around them. The two DNAs approach one another most closely near the bottom of the cleft, where they also approach the catalytic

center of the protomer bound to the opposite DNA, thereby providing a structural explanation for catalysis in *trans*. The cleft, which presumably accommodates target DNA in the final stage of the reaction, appears blocked in some images by a thin bridge of protein that has tentatively been assigned to the enhancer-binding domain of MuA. However, since the enhancer DNA segment is only bound in a transitory fashion during transpososome assembly, it would not be expected to block target binding.

A particularly striking feature of the complex is that it appears to be held together almost entirely by protein–DNA contacts: Significant protein–protein interactions are proposed only at the very bottom of the V. This may provide flexibility to the overall complex, which is known to become increasingly stable with each step of the reaction, presumably via substrate-induced conformational changes. The paucity of protein–protein contacts explains why tetramerization of MuA does not occur in the absence of DNA. In these complexes, a rotation of the lower subunits relative to one another is necessary to bring the DNA ends into juxtaposition with the proposed location of the catalytic centers for the next chemical event, transesterification to a target DNA. It is tempting to speculate that incorporation of the target DNA into the large cleft may provide even more stabilizing protein–DNA contacts that could be used to drive this second reaction forward, perhaps in part by triggering a conformational change that disengages the final displaced 3' hydroxyls from the active sites.

Thus this new model helps explain many previous observations, but also contains some surprising features and makes testable predictions that will certainly drive future biochemical experiments. It is an approach that will surely prove tempting to others stymied by large complexes that do not yield readily to structural methods.

Figure 2. The puzzle pieces of Mu. NMR and X-ray structures have been determined for nearly all of the isolated domains of the 663-residue MuA protein. The minimal active Mu transpososome contains four copies of MuA and two 50-bp DNA segments. However, due to flexible linker regions between domains and the lack of any structures in complex with DNA, it has until now been impossible to assemble these substructures into a model of the transpososome. (Red) The enhancer-binding domain Iα (Clubb et al. 1994); (yellow and green) the Mu end-binding domains Iβ and Iγ (Clubb et al. 1997; Schumacher et al. 1997); (blue) the active-site-containing domain II (Rice and Mizuuchi 1995). The first and last residues of each structure are labeled. Cartoons were made with Ribbons (Carson 1997).

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