MEETING REPORT

PHAGE MU SHEDS *NU* LIGHT ON TOPOISOMERASES

COLD SPRING HARBOR, N.Y.—Topoisomerases are essential for DNA replication and recombination; as such, they are the targets for some of the most effective anticancer drugs—such as adriamycin. Speaking at this year's Cold Spring Harbor meeting on bacterial and phage genetics, Martin Pato (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) presented evidence for the first site-specific activity associated with these important enzymes that coil and relax superhelical DNA. How studies of the mechanism of transposition of coliphage Mu led to uncovering this new activity is an object lesson in the value of continuing to practice phage molecular genetics in this era of biotechnology.

With a length of 37 kilobases (kb), Mu is one of the largest transposable genetic elements. It is also among the most efficient. During a lytic infection, Mu undergoes about 100 rounds of replicative transposition in less than one hour. A few years ago, Pato and his collaborators at the Free University of Brussels (Faelen et al. *Virology* **153:**70, 1986) discovered that insertions of as little as 3 kb in nonessential regions of the genome measurably reduced this frequency, and that larger insertions (50 kb) decreased transposition by four orders of magnitude. To explain this length dependency, he proposed the following model.

It is generally thought that the initial event in transposition involves bringing the ends of the transposing element into close proximity. For most transposons the distance between the ends is relatively small (a few kb); and mechanisms such as protein tracking or random collision could bring them together. In the case of Mu, however, with ends 37 kb apart, Pato postulated a site located near the middle of the Mu genome that could be used to wind up the DNA, thus reducing the distance between the ends. Such a site would have symmetry properties that could explain the previously observed length dependency, and would also predict the effects of other insertions or deletions on transposition, depending on how they altered the symmetry. Those constructions tested thus far are consistent with the predictions of the symmetrical site model. For example, a 10 kb deletion mutant, in which no known transposition genes are affected, is as transposition deficient as a 10 kb insertion mutant.

If the model is correct, what is the nature of the postulated site? A strong DNA gyrase binding region might be a reasonable candidate. *Escherichia coli* DNA gyrase, a type II topoisomerase, supercoils DNA by breaking both strands of the helix—with a four-base stagger between the cuts—allowing a second duplex region to pass through the break before resealing. Pato and his collaborator, Patrick Higgins (Univ. of Alabama, Birmingham), therefore looked for sites in Mu DNA that were particularly susceptible to cleavage by gyrase. Such cleavage sites can be detected by incubating DNA in the presence of gyrase and drugs like oxolinic acid, which prevent resealing, and examining the resulting DNA on denaturing gels.

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When the scientists performed such experiments on fragments of Mu DNA, they saw some indication of a preferential cleavage site in a restriction fragment from the middle region of the genome. Several years ago, however, a peculiar Mu mutant—*nu*B—whose phenotype was an enhanced ability to replicate in gyrase-deficient hosts, had been described (Yoshida et al. *Virology* **120:**269, 1982). Perhaps the *nu*B phenotype resulted from the creation of an even stronger gyrase binding site. When the researchers looked at such mutants using the oxolinic acid system, they saw clear evidence for a strong gyrase cleavage site exactly in the middle of the Mu genome. Cloning and sequencing this region showed that two *nu*B mutant alleles arose from single base changes a few bases away from the cleavage site itself. Interestingly, this very strong binding site, which fulfills many of the criteria for a DNA organizing region, is unrelated to the previously described consensus gyrase-binding sequence.

—Harvey Bialy