The Function of hMSH2-hMSH6 During Mismatch Repair

In their paper, Gradia, et al. address the function of the human MutS homologs hMSH2-hMSH6, which are components of the human mismatch repair (MMR) system. Their studies support the hypothesis that hMSH2-hMSH6 acts as a molecular switch that initially binds mismatched DNA in its ADP-bound state, then forms a sliding clamp that diffuses along the DNA backbone independent of ATP hydrolysis. They characterize hMSH2-hMSH6 binding and release from mismatched DNA using a series of blocking experiments involving streptavidin bound to biotinylated DNA ends. Additional experiments illustrate the need for magnesium and the effects of blocked ends and circular DNA structures on the intrinsic ATPase activity of hMSH2-hMSH6. The paper finishes with an analysis of the structural transformations accompanying adenosine nucleotide binding, showing that ADP-bound hMSH2-hMSH6 differs from other forms in structure.

The results reported strongly support the authors' case for ADP-bound hMSH2-hMSH6 preferential binding of mismatched DNA. Using gel mobility shift assays they demonstrate that the inclusion of ADP in a binding reaction containing hMSH2-hMSH6 and mismatched linear DNA results in a shifted complex specific for mismatched DNA (shown in Figure 1C). They go on to show that the addition of ATP to ADP-bound complex and DNA leads to dissociation of the complex from the DNA following an exchange of ADP→ATP assuming a free DNA end, the exchange presumably occurring as a result of mismatch recognition. Experiments with magnesium further supported this conclusion by showing that ADP-bound hMSH2-hMSH6 could first bind mismatched DNA with or without magnesium present, while binding of the ATP-bound complex requires magnesium atoms (Figure 1E). Later experiments with more

physiologically relevant circular DNA also showed that the ADP-bound complex bound linear and circular plasmid in the same way.

If the authors' model for hMSH2-hMSH6 as a sliding-clamp-like complex is accurate, it should be possible for a single such complex to direct the repair of multiple DNA mismatches present on a single plasmid template. An experiment to test this hypothesis would begin with exposing purified hMSH2-hMSH6 to relatively high concentrations of a plasmid substrate with at least three mutated restriction sites of different types (the result of a single mismatch at each one) in the presence of ADP. Control plasmids missing some of these mismatches also would be prepared. The ratio of complex to plasmid would be very low, as in a template challenge assay (perhaps 1:1000), ensuring that a given plasmid would have at most one complex bound. These plasmids would subsequently be added to solution containing ATP, magnesium, and all the remaining proteins required for MMR *in vitro*. Following incubation, the plasmids could be digested by restriction enzymes corresponding to all three originally mismatched restriction sites, again in the presence of high ATP concentrations (to prevent rebinding of complex). The products would be separated on an agarose gel, and if MMR of multiple sites by one complex was successful, three bands equal to the lengths between restriction sites should be detected on the gel. If the complex is only able to direct repair of one site, a single band the size of the full-length linear plasmid should be observed.