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The Folding of an Immobile DNA-Branched Junction

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ABSTRACT: The central molecular paradigm in recombination is the Holliday junction. This is a structure, in which four strands pair to form four double helical arms that flank a branch point. In the presence of Mg^{2+} , the junction folds into a structure in which the four arms stack on each other pairwise to form two double helical domains. By contrast, in the absence of Mg^{2+} , or when bound to RuvA, a fourfold symmetric structure is seen. The hydroxyl radical cleavage pattern is sensitive to the difference of these two structures. Relative to their pattern in linear duplex DNA, the two crossover strands are protected from attack at sites four nucleotides 3' to the junction in the two-domain structure, because these sites occlude each other. In the absence of Mg^{2+} , these sites are no longer protected, so the folding of this key intermediate is measurable through these differences. Preliminary experiments have revealed that the stacking reaction rate is about 1.6 sec^{-1} , at room temperature. We are currently attempting to get better statistics on this rate, and to measure the influence of temperature on it. We are also attempting to measure the unstacking rate by using EDTA to remove Mg^{2+} . Recently, we have established the $[Mg^{2+}]$ optima for performing the measurements. Future plans also involve measuring the influence of arm-length on the folding rates.