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Characterization and Modification of DNA Polymerases for Use in DNA Sequencing

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Our studies are directed towards improving the properties of DNA polymerases for use in DNA sequencing. The primary focus is understanding the mechanism by which DNA polymerases discriminate against nucleotide analogs, and the mechanism by which they incorporate nucleotides processively without dissociating from the DNA template.

We are comparing three DNA polymerases that have been used extensively for DNA sequencing; *E. coli* DNA polymerase I, T7 DNA polymerase, and Taq DNA polymerase. These are related to one another, and this homology has been exploited to construct active site hybrids that have been used to determine the structural basis for differences in their activities. Specifically, the hybrids have been used (1) to determine why *E. coli* DNA polymerase I and Taq DNA polymerase discriminate strongly against dideoxynucleotides, and (2) to understand how T7 DNA polymerase interacts with its processivity factor, thioredoxin, to confer high processivity.

Based on these studies, we have been able to modify Taq DNA polymerase and *E. coli* DNA polymerase I to make them incorporate dideoxynucleotides much more efficiently, and to have increased processivity in the presence of thioredoxin. The ability to incorporate dideoxynucleotides efficiently greatly improves the uniformity of band intensities on a DNA sequencing gel, thereby increasing the accuracy of the DNA sequence obtained. In addition, the efficient use of dideoxynucleotides reduces the amount of these analogs required for DNA sequencing, an important issue when using fluorescently modified dideoxy terminators. In an approach that complements these studies, we, in collaboration with Dr. Thomas Ellenberger (Harvard Medical School), are determining the crystal structure of T7 DNA polymerase in a complex with thioredoxin and a primer-template. Knowledge of this structure will allow the rationale design of specific mutations that will enable DNA polymerases to incorporate other analogs useful for DNA sequencing more efficiently, such as those with fluorescent moieties on the bases.

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Modular Primers for DNA Sequencing

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We are developing molecular approaches to DNA sequencing enabling primer walking without the step of chemical synthesis of oligonucleotide primers between the walks. One such approach involves "modular primers" described earlier, consisting of 5-mers, 6-mers or 7-mers (selected from a presynthesized library), annealing to the template contiguously with each other. Another approach, that we have termed DENS (Differential Extension with Nucleotide Subsets), works by selectively extending a short primer, making it a long one at the intended site only. DENS starts with a limited initial extension of the primer (at 20-30 C) in the presence of only 2 out of the 4 possible dNTPs. The primer is extended by 6-9 bases or longer at the intended priming site, which is deliberately selected, (as is the two-dNTP set), to maximize the extension length. The subsequent sequencing/termination reaction at 60-65 C then accepts the extended primer at the intended site, but not at alternative sites, where the initial extension