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CONFORMATIONAL COUPLING IN DNA POLYMERASE FIDELITY

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KEYWORDS: DNA replication, DNA error correction, transient kinetics, induced-fit

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PERSPECTIVES

Most enzymes have evolved to possess either a broad substrate specificity in order to accommodate a range of structurally similar substrates or a strict substrate specificity allowing selection of a single substrate against a pool of close homologs. DNA polymerases are remarkable in that they do both by

altering their substrate specificity during each catalytic cycle according to the DNA template. It is even more remarkable that polymerases can accomplish this task with such extraordinary speed and efficiency. For example, T7 DNA polymerase catalyzes correct base pair insertion at rates of 300–500 bases per second with error frequencies of approximately one in 10^5 to 10^6 bases (1, 2). When this polymerase does make a mistake, it stops to correct the error by removing the mismatch for all but one out of 10^3 to 10^4 bases, such that the overall error frequency approaches one in 10^8 to 10^{10} base pairs (3). To determine how the polymerase can achieve such extraordinary fidelity, we must study the structures of the base pairs and the enzymes and understand the kinetics and thermodynamics of the reactions occurring at the enzyme active site.

Three distinct reactions contribute to the overall fidelity of DNA replication in vivo: the polymerization reaction per se, the proofreading exonuclease reaction (4, 5), and the postreplication error repair system (6). This review focuses on the first two reactions, which are properties of the DNA polymerase holoenzyme, by summarizing our current understanding of the structural, kinetic, and thermodynamic basis of DNA polymerase fidelity. This analysis has led to a new paradigm for understanding DNA polymerization based upon two conformational states of the E-DNA complex required for each round of processive synthesis.

Two excellent reviews describing eukaryotic DNA polymerases and the fidelity of DNA replication were published in this series in 1991 (5, 7), and a recent review summarized the relevant data on T4 polymerase (8). Since these reviews have adequately covered the literature prior to 1991, this review focuses on the significant advances made in the past two years in our understanding of the mechanistic basis of DNA polymerase fidelity. We are selective rather than exhaustive in our coverage of the polymerases, so that appropriate focus can be given to those systems for which sufficiently detailed mechanistic information is available. Comparisons are made to other polymerases when possible to illustrate the generality of the conclusions.

ENZYMOLOGY OF DNA REPLICATION

Base-Pairing Free Energies and the Fidelity Problem

The difference in free energy of binding for correct versus incorrect base pairs in solution is not sufficient to account for the selectivity observed for the enzyme-catalyzed polymerase reaction. The free energy difference is defined by the thermodynamic relationship, $\Delta\Delta G = RT\ln(K_c/K_i)$, where K_c and K_i are the binding constants for correct and incorrect nucleotides, respectively.

on measurements of stability of polynucleotide helices containing variable numbers of mismatched bases (4). Even lower estimates resulted when the analysis was based on terminal mispairs (9). The 1–3 kcal/mole free energy difference would lead to an error frequency of one out of 5–150 bases if the polymerase simply zippered together those base pairs that formed a stable complex in solution. This is in contrast to the observed error frequencies for most polymerases, which are in the range of 10^{-3} to 10^{-5} (4, 5).

Analysis of the structures of mispairs by crystallography (10–13) and nuclear magnetic resonance (NMR) (14–17) has been quite revealing by pointing to ways in which enzymes might selectively destabilize mismatched base pairs relative to the structures observed in aqueous solution. The most remarkable property of the G:C and A:T base pairs is their geometric equivalence and the pseudo twofold axis of symmetry in the plane of the base pairs. As illustrated by the structure of the G:T mismatch shown in Figure 1 (10), the Watson-Crick base pair geometry is violated in forming the mismatched base pair, with the thymine projecting into the major groove and the guanine projecting into the minor groove. However, there is very little local perturbation of the helix and, more importantly, the global conformation of the duplex is unaffected. Similar results have been reported for the A:C mispair (12). Secondly, the structures of mismatched bases reveal water molecules that hydrogen bond to any unsatisfied hydrogen bond donors or acceptors in the mismatched bases.

At the outset, we are not restricted in our analysis of fidelity by the

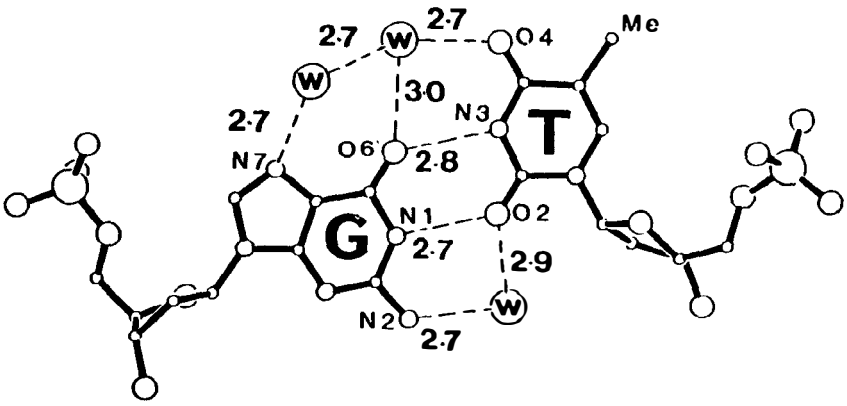

Figure 1 Guanine-Thymine mismatch structure. This structure shows the wobble of the base pairs and the locations of hydrogen-bonded water molecules in the G:T mismatch. The thymine projects into the major groove while guanine projects into the minor groove of the DNA helix. Atoms involved in hydrogen bonds are labeled. The numbers give the lengths of the hydrogen

magnitude of the selectivity as it appears in solution. Rather, we are faced with the task of specifying the mechanisms by which the free energy differences between right and wrong bases are enhanced by the polymerase and utilized in translating nucleotide binding energy into catalytic efficiency. Thus, it is conceivable that the enzyme could reduce the free energy of mismatch formation by excluding water from the active site; this would effectively subtract the thermodynamic contribution of the hydrogen-bonded water molecules seen in the mispairs in solution. In addition, the enzyme could select against mispairs by maintaining an active-site surface contour that restricts the geometry of the base pairs. The intrinsic binding energy from interactions with the ribose and triphosphate portions of the deoxynucleoside triphosphate (dNTP) could be utilized to assist in the desolvation and alignment of the bases in the proper orientation. The details of how this is accomplished must be addressed by direct measurement of the individual steps of the polymerization reaction. However, conceptually, one is faced with the problem of rapidly binding dNTP from solution while maintaining a highly restricted active-site topology.

Selectivity is defined by the enzymatic specificity constant, k_{cat}/K_m for the correct versus incorrect base pairs. These steady-state kinetic parameters define the relative rates of catalysis for incorporation of correct versus incorrect base pairs, weighted by the concentrations of the corresponding base pairs.

$$\text{Relative rates} = \frac{(k_{cat}\ K_m)_{correct} \times [\text{correct}]}{(k_{cat}\ K_m)_{incorrect} \times [\text{incorrect}]} \qquad 1.$$

Although the steady-state kinetic parameter, k_{cat}/K_m, defines the selectivity, resolution of the relative contributions of k_{cat} and K_m towards fidelity does not provide sufficient information to establish the mechanistic basis for fidelity. Rather, resolution of the elementary steps in the reaction sequence and their contributions towards the observed selectivity is necessary to understand the mechanisms by which the enzymes achieve such extraordinary fidelity.

Properties of Simple DNA Polymerases

Although quite a large number of polymerases have now been purified and characterized from various sources (7, 8, 18–23), only the simplest of the polymerases have lent themselves to the detailed investigations necessary to examine fidelity at the mechanistic level. Polymerases that are involved in replication of genomic DNA consist of a complex of several proteins that are assembled in a highly controlled manner as a necessary part of cellular

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