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How DNA Polymerases Catalyze DNA Replication, Repair, and Mutation

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Despite more than half a decade of active research, new intermediate structures, mechanistic features, and biological functions have continued to be uncovered for DNA polymerases (pols), and such activities have intensified in recent years. To provide an update of these developments, particularly in the aspects of structure and mechanism, this issue of *Biochemistry* presents a collection of five timely Current Topics in *Biochemistry*.[1−5] While these five articles emphasize specific pols or specific families of pols, this Perspective raises some of the common issues, as well as some recent developments not covered in the five Current Topics.

SIMILAR CATALYTIC DOMAIN STRUCTURES WITH DIVERSE FIDELITY, MECHANISM, AND FUNCTION

As the name suggests, the function of DNA polymerases is to catalyze the polymerization of DNA with very high fidelity. However, many new DNA polymerases with lower fidelity (i.e., error-prone) have been discovered in the past 15 years. The low-fidelity polymerases have many important biological functions ranging from various types of DNA repair to translesion synthesis, V(D)J recombination, and somatic hypermutation.[6,7] These pols have been classified into seven families on the basis of sequence homology: A−D, X, Y, and reverse transcriptase.[6] Pols usually consist of a "catalytic domain" and often contain additional domains for specific functions, such as an exonuclease domain, a BRCT domain, an 8 kDa domain, a PAD domain, etc. The catalytic domain can be further dissected into palm, thumb, and fingers subdomains for most pols, except that pol X from African swine fever virus (ASFV) consists of only palm and fingers subdomains.[8,9] As described in this collection of Current Topics in *Biochemistry*, different families of pols, or even different pols within the same family, differ substantially in their fidelities and mechanisms of fidelity. Thus, a major question in polymerase research is how relatively small differences in the core structure confer substantial differences in fidelity, mechanism, and biological function.

HOW DNA POLYMERASES CATALYZE WATSON−CRICK INCORPORATIONS

Like other enzymes, the key mechanistic issues are how the polymerase achieves its "substrate specificity" and "rate enhancement". However, while most other enzymes control substrate specificity mainly by the enzyme, DNA polymerases typically bind DNA first, and the bound DNA then plays a major role in dNTP specificity via Watson−Crick base pairing. This mechanistic feature can explain that, while most other enzymes have evolved for one optimal substrate, pols have four optimal dNTP substrates and rely on the DNA to choose one in each catalytic cycle. It also allows pols responsible for DNA synthesis to achieve very high fidelity. However, this textbook version of the mechanism has also generated heated debate on several issues. Some of the recent developments are summarized below.

(a) Is hydrogen bonding required for Watson−Crick base pairing? It is well established that a broad range of dNTP analogues, including some hydrophobic ones, can be incorporated into DNA by some pols, suggesting that hydrogen bonding in the Watson−Crick base pairs is not important for polymerase catalysis.[10] On the other hand, detailed pre-steady state kinetic study showed that hydrogen bonding in the Watson−Crick base pair is important in pol catalysis.[1,11] It is likely that the different conclusions from different reports were due to different methods of analyses and/or different pols being studied. Recently, Romesberg, Marx, and co-workers showed that, despite the absence of Watson−Crick-like hydrogen bonds, and unlike in duplex DNA, the structure of the dNaM:d5SICSTP unnatural base pair in the KlenTaq polymerase active site is similar to that of a natural base pair.[12]

(b) Is Watson−Crick base pairing sufficient to account for high fidelity? The early concept was that the energetic difference between Watson−Crick pairing and mismatches is too small to fully account for the very low error rate (one per 10^4−10^6) of high-fidelity pols;[13] thus, the enzyme also contributes to the selection of the correct dNTP. The mechanism for the latter was a major question of research for decades. An early theory suggested that the dNTP-induced conformational closing was the rate-limiting step, thus contributing to the fidelity.[13] However, this conformational change was later shown to be non-rate-limiting for pol β[14] and other pols, and later reports indicated that some pols pre-exist in the closed conformation before binding of MgdNTP.[15−17] This then raised two additional questions: what is the rate-limiting step, and can a non-rate-limiting step also contribute to fidelity? These issues have been the subjects of very active research in the past decade as described in most of the Current Topics in this issue and in other recent reports.[18−20] Despite such extensive research, contrasting views still exist,[21,22] and new insights continue to be uncovered. Recently, Kuchta reported that direct measurements of the energetic difference between the synthesis of correct and incorrect base pairs found it to be much larger than previously believed and to be sufficient to account for the high fidelity of DNA replication.[23] Thus, pols may have evolved sophisticated mechanisms for kinetic rather than thermodynamic reasons. On another front,

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McKenna and Goodman reported an interesting correlation between the strength of Watson−Crick H-bonding and base stacking interactions in the active site and transition state stabilization energy, on the basis of Brønsted plots of the catalytic rate constant versus pK_a for the leaving group.[24] Even though chemistry as the rate-limiting step has been established previously for pol β at least,[2,5] this work provided the first direct evidence that stabilizing interactions mediated by nucleotide base pairing in the active site perturb the structure of the transition state. The results also support an early proposal that "fidelity is derived fundamentally from differential substrate binding at the transition state of a rate-limiting chemical step".[25]

(c) Structures of intermediate complexes. An important approach to resolving the controversial issues of which intermediate steps in the catalytic cycle are important for the fidelity is to elucidate the relationship between the rates of formation of kinetic intermediates and their structures. For each pol, researchers have tried to determine structures of the intermediates identified in various kinetic studies. Hundreds of such structures have already been reported, some of which are summarized in four of the Current Topics in this collection.[1−4] In addition, other approaches such as single-molecule Förster resonance energy transfer,[19,20,26] molecular dynamic simulation,[27] and small-angle X-ray scattering[28] have also been used to characterize the intermediates. A highly significant recent development is the use of time-resolved X-ray crystallography to determine the structures of intermediates for pol η and pol β as described in the respective Current Topics.[2,4]

HOW DNA POLYMERASES CATALYZE NON-WATSON−CRICK INCORPORATIONS

A number of low-fidelity pols catalyze translesion synthesis and/or non-Watson−Crick incorporations. The structural mechanism of translesion synthesis has been studied extensively and described in some of the Current Topics in this collection. The mechanism of non-Watson−Crick incorporation is important for understanding not only how low-fidelity pols overcome Watson−Crick base pairing to achieve low fidelity but also how high-fidelity pols make errors, however rarely. Though many structures of mismatched ternary complexes have been reported, the incoming MgdNTP does not form a nascent pair with the template base in most cases, possibly because the structures with a "nascent mismatch pair" are unfavorable for crystallization. One strategy for overcoming this problem is to use lower-fidelity mutants as in a pol λ mutant ternary complex with dT:dGMPPCP[29] and a RB69 mutant complex with all 12 mismatches,[30] or a lower-fidelity metal ion (replacing Mg^{2+} with Mn^{2+}) as in the dC:dATP complex of the *Bacillus stearothermophilus* pol I large fragment.[31] These studies suggested that pols can make errors via rare tautomers that mimic the shape of Watson−Crick pairs and provided structural insight into how RB69 achieves its high fidelity.

Another strategy is to use nuclear magnetic resonance (NMR) to determine the solution structure, which has found success for the ASFV pol X complex with a dG:dGTP mismatch.[32] This polymerase catalyzes efficient dG:dGTP incorporation in addition to correct repair.[33] NMR and binding studies also showed that ASFV pol X can prebind purine MgdNTP tightly in an unusual *syn* conformation stabilized by partial ring stacking with His115. When a gapped DNA binds, the prebound *syn*-dGTP forms a Hoogsteen base pair with the template *anti*-dG.[32] These and related studies suggested that ASFV pol X uses two different mechanisms to catalyze dNTP incorporations: the canonical mechanism that does not involve His115 and the dNTP prebinding mechanism for dG:dGTP mediated by His115. It will be interesting to examine if this "dual-mechanism model" can also be applied to other low-fidelity pols.

FUTURE PERSPECTIVES

It appears that the research in the structure and mechanism of DNA polymerases will continue to be highly active for the foreseeable future. In particular, understanding the role of protein dynamics in the polymerase fidelity has been mentioned as an important future direction in some of the Current Topics. As a result of such active research, we will not only learn about the structure and mechanism of DNA polymerases but also uncover new principles of enzyme catalysis. Another direction, which has been rarely explored, is the effect of proteins that associate with the polymerase *in vivo* on the structure and mechanism of pols. Only when we move to that stage will we be able to fully understand how each particular pol plays its specific biological role.

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Notes

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