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Minireview

DNA polymerases involved in the incorporation of oxidized nucleotides into DNA: Their efficiency and template base preference

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ABSTRACT

Genetic information must be duplicated with precision and accurately passed on to daughter cells and later generations. In order to achieve this goal, DNA polymerases (Pols) have to faithfully execute DNA synthesis during chromosome replication and repair. However, the conditions under which Pols synthesize DNA are not always optimal; the template DNA can be damaged by various endogenous and exogenous genotoxic agents including reactive oxygen species (ROS), and ROS oxidize dNTPs in the nucleotide pool from which Pols elongate DNA strands. Both damaged DNA and oxidized dNTPs interfere with faithful DNA synthesis by Pols, inducing various cellular abnormalities, such as mutations, cancer, neurological diseases, and cellular senescence. In this review, we focus on the process by which Pols incorporate oxidized dNTPs into DNA and compare the properties of Pols: efficiency, i.e., k_{cat}/K_m, k_{pol}/K_d or V_{max}/K_m, and template base preference for the incorporation of 8-oxo-dGTP, an oxidized form of dGTP. In general, Pols involved in chromosome replication, the A- and B-family Pols, are resistant to the incorporation of 8-oxo-dGTP, whereas Pols involved in repair and/or translesion synthesis, the X- and Y-family Pols, incorporate nucleotides in a relatively efficient manner and tend to incorporate it opposite template dA rather than template dC, though there are several exceptions. We discuss the molecular mechanisms by which Pols exhibit different template base preferences for the incorporation of 8-oxo-dGTP and how Pols are involved in the induction of mutations via the incorporation of oxidized nucleotides under oxidative stress.

Contents

1. Introduction

Chromosome DNA is continuously exposed to various endogenous and exogenous genotoxic agents. Among these agents, oxidation is one of the most common threats to genetic stability [1,2]. Each human cell is estimated to metabolize approximately 10^{12} molecules of oxygen per day, and approximately 1% of oxygen

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metabolism results in the production of reactive oxygen species (ROS) via one electron reduction [3,4]. These reactive molecules include superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, with hydroxyl radicals thought to be the most predominant reactive species [5]. ROS are also generated in cells when they are exposed to radiation and chemical carcinogens. To counteract the oxidative stress induced by ROS, cells have evolved multiple defense mechanisms. Enzymes, such as catalase or superoxide dismutase, detoxify ROS and low-molecular-weight scavengers, such as glutathione, reduce the toxicity of ROS [6]. Nevertheless, some ROS molecules escape from the defense systems and inevitably damage the bio-molecules. Thus, ROS have been implicated in the etiology of human degenerative diseases, aging, and cancer [7,8].

Although ROS generate a variety of modified bases in DNA, 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) is the best characterized oxidized base in chemistry and biology [9–11] and is used as a biomarker of DNA oxidation. Approximately 10^3 8-oxo-dG molecules are generated in normal human cells per day [2,12]. 8-oxo-dG pairs with cytosine in the *anti* conformation but assume the *syn* conformation when pairing with adenine [13–15]. In fact, both dATP and dCTP are inserted opposite template 8-oxo-dG during DNA synthesis [16]. The latter pairing can result in G:C to T:A transversion [17]. To prevent the mutagenesis caused by the lesion, human cells possess multiple repair mechanisms [18], including DNA glycosylases in the base excision repair pathway, such as 8-oxoguanine glycosylase (OGG1) and MutY glycosylase homologue (MUTYH), which excise 8-oxo-G when paired with cytosine and adenine opposite 8-oxo-dG, respectively [19]. The Cockayne syndrome proteins CSA and CSB in transcription-coupled nucleotide excision repair also involve the exclusion of 8-oxo-dG from DNA [20–22]. Despite the presence of repair mechanisms, 8-oxo-dG accumulates in senescent cells and the brain cortex of aged humans, which may cause various cellular abnormalities [23–26].

In addition to the direct oxidation of deoxyguanosine (dG) in DNA, 8-oxo-dG can be generated by the incorporation of oxidized dGTP (8-oxo-dGTP) into DNA by DNA polymerases (Pols). 8-oxo-dGTP can be incorporated into the template strand opposite deoxycytidine (dC) or deoxyadenosine (dA) and the latter may cause A:T to C:G transversions [27]. In fact, *Escherichia coli mutT* mutants, which lack the ability to hydrolyze 8-oxo-dGTP to its mono-phosphate form, exhibit more than 1000 times higher frequencies of spontaneous A:T to C:G transversions than controls [28]. Mice lacking MTH1, a mammalian homologue of MutT, display enhanced tumor formation in the lung, liver, and stomach [29]. In human cells, suppression of MTH1 expression induces cellular senescence [30]. In contrast, the over-expression of hMTH1 reduces total cellular 8-oxo-dG levels in human cells and transgenic mice [31,32]. Over-expression also suppresses genome instability in cells with defective mismatch repair (MMR) mechanisms and causes ameliorated neuropathological and behavioral symptoms resembling Huntington's disease in mice. Thus, the oxidized dNTP pool is recognized as a source of spontaneous mutagenesis, carcinogenesis, cellular senescence, and neurological disease.

In human cells, MTH1 hydrolyzes 8-oxo-dGTP and other oxidized dNTPs, such as 2-hydroxy-dATP (2-OH-dATP) and 8-oxo-dATP, to the mono-phosphate forms in the nucleotide pool [33]. In addition to MTH1, cells possess MTH2, which hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP [34], and Nudix type 5 (NUDT5) protein, which hydrolyzes 8-oxo-dGDP to the mono-phosphate [35]. MMR prevents the mutations caused by the incorporation of 8-oxo-dGTP [31]. 8-oxo-dG incorporated during replication can become a target of MMR machinery, which removes the incorporated 8-oxo-dG from DNA and initiates DNA re-synthesis.

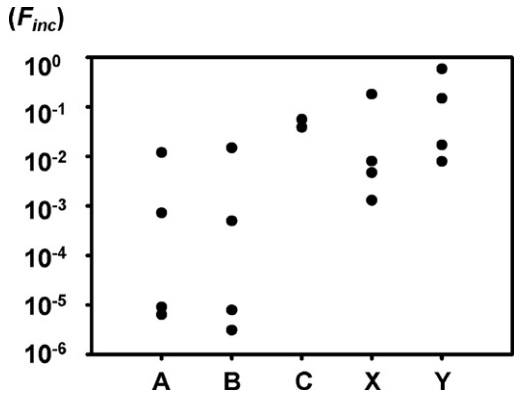

Fig. 1. Efficiency of the incorporation of 8-oxo-dGTP into DNA by family A, B, C, X, and Y Pols. F_{inc} indicates the ratio of the efficiency of incorporating 8-oxo-dGTP opposite a favorable template base versus that of incorporating normal dNTPs opposite the same template base. F_{inc} was calculated from the kinetic parameters in the references (see Table 1). Pols include hPolγ, *E. coli* Pol I Klenow fragment (exo⁻), and T7 Pol for family A; hPolα, bovine Polδ, *E. coli* Pol II, and φ29 Pol for family B; *E. coli* Pol III* and α subunit of Pol III for family C; hPolβ, hPolλ, and African swine fever virus Pol X for family X; and hPolη, Polι, and Polκ for family Y.

Measurements of intracellular levels of 8-oxo-dGTP in nucleotide pool are a challenging issue. Initial attempt to measure the level of 8-oxo-dGTP in *E. coli* was failed [36]. Recent measurements with improved sensitive methods indicate that the level of 8-oxo-dGTP in mitochondria is similar to that of normal dTTP, which is 1–10% of the level of normal dGTP [37]. It remains to be seen the levels of 8-oxo-dGTP and other oxidized dNTPs in nucleus in oxidative stressed and non-stressed human cells.

To exert adverse effects, oxidized dNTPs must be incorporated into DNA by Pols. In culture medium, 8-oxo-dG is readily incorporated into the genomic DNA of human cells upon phosphorylation [38]. As shown below, however, various Pols have distinct properties in terms of their efficiency in incorporating 8-oxo-dGTP into DNA. In this review, we use the term "efficiency" to mean k_{cat}/K_m or V_{max}/K_m in steady-state kinetic analyses and k_{pol}/K_d in pre-steady-state kinetic analyses. Some Pols incorporate 8-oxo-dGTP into DNA very efficiently, whereas others do so poorly. So far, we have surveyed the maximum difference of F_{inc} (i.e. the ratio of the efficiency for incorporating 8-oxo-dGTP versus that of incorporating normal dNTP) among various Pols to be 10^5 (Fig. 1, Table 1). For example, human Polη incorporates 8-oxo-dGTP opposite template dA with 20–60% efficiency compared to normal dTTP incorporation [39,40], whereas *E. coli* Pol II exo⁻ incorporates it opposite template dC with 0.0003% efficiency compared to normal dGTP incorporation [41]. In addition, the preference of the Pols for template bases is distinct (Table 2); Polη incorporates 8-oxo-dGTP opposite template dA almost exclusively [40], and *E. coli* Pol II exo⁻ incorporates it opposite template dC more favorably (the ratio for incorporation opposite template dC:dA is 22:1) [41].

In this review, we compare the relative efficiency and template base preferences of Pols for incorporating 8-oxo-dGTP. Because Pols are categorized into six families (A, B, C, D, X, and Y) based on their primary structures [42], we review the properties of Pols family by family. The A, B, or C families of Pols are involved in chromosome replication with high fidelity and high processivity, and the X and Y families are responsible for DNA repair synthesis and translesion synthesis (TLS). Pols family D is restricted to Archaea, and their properties for 8-oxo-dGTP incorporation have not been reported. Therefore, we omit the D-family Pols from this review. We also discuss mechanisms underlying the distinct properties of some Pols and the biological consequences of the incorporation of 8-oxo-dGTP.