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Insights into the glycosylase search for damage from single-molecule fluorescence microscopy

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ABSTRACT

The first step of base excision repair utilizes glycosylase enzymes to find damage within a genome. A persistent question in the field of DNA repair is how glycosylases interact with DNA to specifically find and excise target damaged bases with high efficiency and specificity. Ensemble studies have indicated that glycosylase enzymes rely upon both sliding and distributive modes of search, but ensemble methods are limited in their ability to directly observe these modes. Here we review insights into glycosylase scanning behavior gathered through single-molecule fluorescence studies of enzyme interactions with DNA and provide a context for these results in relation to ensemble experiments.

1. Introduction

1.1. Base excision repair

Endogenously produced DNA damages, including oxidative damages, are repaired by the base excision repair (BER) pathway (for reviews see [1–6]). Base excision repair is comprised of five enzymatic steps and is initiated by DNA glycosylases, enzymes which locate and excise a single damaged base leaving an abasic (AP) site in the DNA backbone. The next step in the pathway is cleavage of the DNA strand 5′ to the AP site by an AP endonuclease. Alternatively, DNA glycosylases that remove oxidized DNA bases contain a lyase activity that cleaves the DNA backbone leaving a sugar or a phosphate group attached to the 3′ end that needs to be removed by a phosphodiesterase or phosphatase activity. After these initial steps, downstream enzymes, including a DNA polymerase and a ligase, work to fill the single nucleotide gap. In mammalian cells this is called short patch repair.

Glycosylases are capable of specifically locating a single damaged DNA base among a sea of undamaged bases. The glycosylase search process involves non-covalent contacts between the enzyme and DNA and utilizes thermal energy to provide a driving force for movement along DNA. One lingering question is how glycosylase enzymes bind and move along DNA to enact an efficient search for damage.

1.2. Insights into the glycosylase search for damage from ensemble studies

Early studies of the Lac repressor protein led to the suggestion that DNA-binding proteins may interact with DNA in a process known as “facilitated diffusion” [7–9]. The existence of facilitated diffusion has been supported by the observation that certain transcription factors and other DNA binding proteins are able to find binding sites at a rate that is faster than would be predicted from a simple three-dimensional diffusive search. A reasonable explanation for the enhanced binding rates is that the DNA is “facilitating” the search by serving as a conduit along which a protein molecule can travel between sites. The simplest interpretation of facilitated diffusion would be sliding along the DNA backbone. Facilitated diffusion is typically observed at low salt concentrations that enhance the electrostatic interactions between proteins and DNA, and these conditions are unlikely to be biologically relevant. However,

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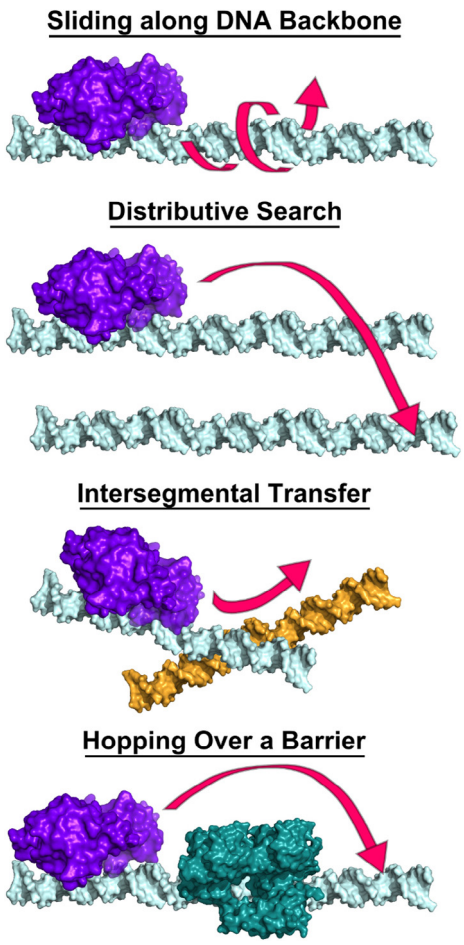

Fig. 1. Some possible mechanisms for glycosylase diffusion on DNA.

facilitated diffusion by the Lac repressor has recently been reported *in vivo* on bacterial chromosomal DNA [10]. Thus, the experimental evidence indicates that facilitated diffusion plays some role in how DNA-search proteins interact with DNA. A precise search mechanism has yet to be resolved, and the relationship between facilitated diffusion and random diffusion has served as an active area of research for over 30 years.

The mechanism by which a glycosylase scans DNA to find damage is likely complicated. From a theoretical standpoint, a glycosylase search mechanism that utilizes only sliding along the backbone is difficult to justify. A particle that moves randomly in one dimension is predicted to remain confined to a region close to its initial position [11,12]. Thus, a sliding search of DNA would be too redundant for the efficient search of an entire genome. Similarly, a purely three-dimensional diffusive search of DNA would be inadequate [8]. A "distributive search" is often defined as dissociation from one DNA molecule followed by three-dimensional diffusion and binding to another molecule (Fig. 1). In the distributive search model, in order for catalysis to take place, the enzyme would have to collide with the damaged substrate in an orientation that facilitates promotion to a reaction transition state. While it has been shown to be theoretically feasible for a glycosylase to rely upon a distributive search to find the small number of damage sites in the nucleus within a reasonable time frame, the likelihood that the enzyme could selectively find them in the presence of a vast excess of undamaged competitor DNA is low [13]. A combination of sliding and distributive interactions seems likely, and facilitated diffusion may employ other search modes. One such mode, known as "hopping," involves dissociation and rebinding to a nearby location on the same DNA molecule (Fig. 1). Hopping can be considered a hybrid between a sliding and a distributive interaction because the enzyme binds to a close location within the same DNA strand [8]. Another possible method of glycosylase travel is "intersegmental transfer," wherein DNA secondary structure facilitates glycosylase relocation to a very distant location on the same strand or to new DNA strand. It has been suggested that glycosylases may use all of these various types of interactions with DNA to find lesions (discussed in [8] and reviewed in [12]).

Early ensemble experiments to test the mechanism of glycosylase search on DNA revealed evidence that glycosylase enzymes employ facilitated diffusion. These experiments investigated T4 endonuclease V repair of UV-induced pyrimidine dimers on plasmid DNA. Lloyd and coworkers created varying numbers of damage sites in plasmid DNA and monitored the accumulation of linear DNA products after incubation with T4 endonuclease V [14]. When limited numbers of enzymes interacted with plasmids that contained several damage sites, the rate of cleavage within a single plasmid exceeded the rate of cleavage among different plasmids. Therefore, the cleavage of sites within a single plasmid was "correlated." These results led to the suggestion that the encounter and enzymatic cleavage of sites was processive, which would imply facilitated diffusion between damage sites. Similar to the Lac repressor experiments, these T4 endonuclease V correlated cleavage experiments were carried out at very low salt, which would enhance glycosylase interactions with DNA. Interestingly, the observation of processive cleavage in T4 endonuclease V preceded confirmation that it was a bifunctional enzyme capable of both glycosylase and lyase activities!

Correlated cleavage experiments also provided evidence for a distributive search mode. When the processive mechanism of T4 endonuclease V was tested using the ensemble plasmid assay at increasing salt conditions, it was found that processivity was highly salt dependent [15,16]. At salt concentrations below 50 mM, correlated cleavage was detected. When the NaCl concentration was above 50 mM, each glycosylase was able to repair several plasmid molecules, indicating a switch to a distributive search. This left the question of whether facilitated diffusion was physiologically relevant for glycosylase enzymes. In order to determine whether a processive mechanism could occur *in vivo*, T4 endonuclease V activity was examined in irradiated *Escherichia coli* cells [17,18]. The first study confirmed that correlated cleavage could occur in the *in vivo* salt and crowding environment of the bacterium by monitoring T4 endonuclease V activity on plasmids within a repair-deficient strain of *E. coli* [17]. The second study introduced enzymatically active, scanning-deficient T4 endonuclease V variants into the repair-deficient strain of *E. coli*. Later structural studies showed that one of the mutated residues (Arg26) is in the catalytic site, while the other residue (Lys33) is within Van der Waals radius of the backbone (PDB: 1VAS; PDB: 2FCC) [19,20]. These variants had lower processivity *in vitro*, and were unable to effectively protect against UV-damage *in vivo* [18]. These studies showed that correlated cleavage could occur within the cell and indicated that a processive search was necessary for cell survival.

Ensemble correlated cleavage studies utilizing linear DNA oligodeoxyribonucleotides (oligos) that contain engineered damages have allowed for the study of glycosylase enzymes that recognize oxidative lesions, methylated sites, and DNA mismatches. These experiments measure the rate at which a glycosylase cleaves consecutive damage sites within a single DNA strand of a duplex. The processivity of *E. coli* uracil DNA glycosylase (Udg) was examined using both plasmid [21] and linear DNA substrates [22–25]. Similar to what was observed for T4 endonuclease V, the processivity of Udg appears to be highly dependent on

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