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Review

Investigations of pyrimidine dimer glycosylases — a paradigm for DNA base excision repair enzymology

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Abstract

The most prevalent forms of cancer in humans are the non-melanoma skin cancers, with over a million new cases diagnosed in the United States annually. The portions of the body where these cancers arise are almost exclusively on the most heavily sun-exposed tissues. It is now well established that exposure to ultraviolet light (UV) causes not only damage to DNA that subsequently generates mutations and a transformed phenotype, but also UV-induced immunosuppression. Human cells have only one mechanism to remove the UV-induced dipyrimidine DNA photoproducts: nucleotide excision repair (NER). However, simpler organisms such as bacteria, bacteriophages and some eukaryotic viruses contain up to three distinct mechanisms to initiate the repair of UV-induced dipyrimidine adducts: NER, base excision repair (BER) and photoreversal. This review will focus on the biology and the mechanisms of DNA glycosylase/AP lyases that initiate BER of *cis–syn* cyclobutane pyrimidine dimers. One of these enzymes, the T4 pyrimidine dimer glycosylase (T4-pdg), formerly known as T4 endonuclease V has served as a model in the study of this entire class of enzymes. It was the first DNA repair enzyme: (1) for which a biologically significant processive nicking activity was demonstrated; (2) to have its active site determined, (3) to have its crystal structure solved, (4) to be shown to carry out nucleotide flipping, and (5) to be used in human clinical trials for disease prevention.

Keywords: T4 pyrimidine dimer glycosylase; DNA base excision repair; UV light; DNA–protein covalent intermediates; Processivity; Nucleotide flipping

Contents

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Prologue

As a graduate student working on the mechanism by which the anticancer drug, bleomycin damaged DNA, my research interests became focused on how cells repair these lesions. With the encouragement of my committee, nine months prior to defending my Ph.D. Dissertation, I contacted Dr. Philip C. Hanawalt concerning the possibility of joining his group. To my surprise, he said that I was welcome to come but ... bring my own money and my own project. Since I was interested in both nucleotide and base excision repair, and since cloning was just being reduced to common practice, I suggested two projects that combined those interests: cloning and enzyme characterization of either the UvrABC genes or the gene encoding T4 endonuclease V. He indicated that there was an extremely talented postdoctoral fellow by the name of Dr. Aziz Sancar in the laboratory of Dr. Dean Rupp at Yale who was already well on his way to cloning the UvrABC genes, and maybe I should go after the T4 endonuclease V. With that one sentence, at least the next 25 years of my career were set, and I will never regret it.

From my prospective, Phil continuously exemplifies the very best that our profession has to offer: the highest ideals and standards, exacting science, the ability to see the very best in people, perseverance through hard times, generosity to his students and postdocs, care in teaching and most of all, a continuous joy derived from discovery of nature's secrets and the sense that being a researcher is the highest of privileges. My time in Phil's lab forever transformed my attitudes about science and he has always been much more than a postdoctoral mentor, but rather he has served as a role model from which to run my own laboratory.

1. Discovery of enzymes that initiate repair of *cis–syn* cyclobutane pyrimidine dimers

The initial description of enzymes that are capable of conferring enhanced resistant to UV killing, came in 1947 from Luria, who reported that the bacteriophage T4 was approximately twice as resistant to UV inactivation as other T-even bacteriophage [1]. The gene that encodes the T4 enzyme was originally termed the *v*-gene and was later named the *denV* gene, referring to *D*NA *en*donuclease V [2,3]. Follow-up studies revealed that the product of the *denV* gene was responsible for the initiation of repair of cyclobutane pyrimidine dimers, incising DNA on the 5′ side of the lesion [4–8]. Similar activities were also described from extracts of the organism *Micrococcus luteus* and these enzymes were named UV correndonuclease I, II and III [9–11]. These original investigations recognized that UV-irradiated DNA was not degraded to nucleotides or dinucleotides, such as is observed with exonucleolytic enzymatic activities, but rather that the DNA was incised within the duplex. Hence, the term endonuclease was used to describe all of these enzymes. Unfortunately, the choice of the biochemical descriptor, "endonuclease" was later proven to be mechanistically incorrect. Investigations from the laboratories of Drs. Haseltine, Grossman, Friedberg, Sekiguchi and Hanawalt all determined that these enzymes functioned as DNA glycosylases that also have a concomitant AP lyase activity [12–17]. This biochemical mechanism has since been rigorously established and thus, these enzymes should no longer be referred to as endonucleases but as DNA glycosylase/AP lyases.

Although the vast majority of the early literature focused on characterizing the enzymatic activities from either bacteriophage T4-infected *E. coli* or extracts from *M. luteus*, more recently many microor-