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Micro-irradiation tools to visualize base excision repair and single-strand break repair

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ABSTRACT

Microscopy and micro-irradiation imaging techniques have significantly advanced our knowledge of DNA damage tolerance and the assembly of DNA repair proteins at the sites of damage. While these tools have been extensively applied to the study of nucleotide excision repair and double-strand break repair, their application to the repair of oxidatively-induced base lesions and single-strand breaks is just beginning to yield new insights. This review will focus on examining micro-irradiation techniques reported to create base lesions and single-strand breaks; these lesions are considered to be primarily addressed by proteins involved in the base excision repair (BER) pathway. By examining conditions for generating these DNA lesions and reviewing information on the assembly and dissociation of repair complexes at the induced lesion sites, we hope to promote further investigations into BER and to stimulate further development and enhancement of these techniques for the study of BER.

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Abbreviations: DIQ, 1,5-dihydroxyisoquinoline; 4-AN, 4-amino-1,8-naphthalimide; 6,4PPs, 6,4-photoproducts; 8-oxodG, 8-oxo-2′-deoxyguanosine; OGG1, 8-oxoguanine glycosylase 1; APE1, AP-endonuclease; ATM, ataxia telangiectasia-mutated gene; BER, base excision repair; BRCA1, breast cancer gene 1; BRCT1, BRCA1C-terminus 1; BrdU, bromodeoxyuridine; CALI, Chromophore-assisted laser or light inactivation; CPD, cyclobutane pyrimidine dimers; dRP, deoxyribose phosphate; DSB, double-strand break; LIGI, DNA ligase I; LIGIII, DNA Ligase III; Pol β, DNA polymerase β; NEIL1, endonuclease VIII-like 1; NEIL2, endonuclease VIII-like 2; NTH1, endonuclease III-like protein 1; EtBr, ethidium bromide; FEN1, flap endonuclease 1; FlAsH, fluorescein arsenical hairpin; FUS/TLS, fused-in-sarcoma/translocated-in-sarcoma; γH2AX, gammaH2AX; GFP, green fluorescence protein; HP1, heterochromatin protein 1; HMGN1, high mobility group N1; H_2O_2, hydrogen peroxide; ICLs, interstrand cross-links; MED1, mediator complex subunit 1; MEFs, mouse embryonic fibroblasts; NAC, N-acetyl-cysteine; ns, nanosecond; NIR, Near Infrared; NBS1, Nijmegen breakage syndrome 1; NER, nucleotide excision repair; CAF1-p150, p150 subunit of chromatin assembly factor I; PAR, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP-1, poly(ADP-ribose) polymerase 1; γH2AX, phosphorylated H2AX; PNKP, polynucleotide kinase phosphatase; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; ROS, reactive oxygen species; SSBs, single-strand breaks; TRE, tetracycline response element; tetR, tetracycline repressor; TA, transcription activator tetR+VP16; TDP1, tyrosyl-DNA phosphodiesterase 1; (UV), ultraviolet light; XRCC1, X-ray repair cross-complementing protein 1; (YFP), yellow fluorescent protein.

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1. Introduction

Genomic DNA is attacked by a number of endogenous and exogenous agents that create base damage and both single- and double-strand breaks. These heterogeneous DNA damaged substrates require highly coordinated repair mechanisms to ensure genomic integrity and cell survival. DNA repair proteins sense damage sites, signal the recruitment of specific repair proteins, and initiate repair. Numerous biochemical and biophysical studies over the past 50 years have provided insight into the mechanism by which DNA damage is sensed and repaired; however, a temporal description of the assembly of DNA repair proteins at the sites of damage is lacking. The diversity of DNA lesions and DNA repair pathways, some with overlapping substrate specificities, has left a number of open questions about how the repair proteins assemble/disassemble to coordinate their activities to efficiently repair disparate DNA lesions.

Fluorescent microscopy has emerged as a powerful technique to visualize the response of DNA repair proteins to damage. Detection of damaged-induced DNA repair foci from globally applied agents, such as ultraviolet light (UV-A, 320–400 nm), ionizing radiation, hydrogen peroxide (H_2O_2), or chemotherapeutics, has been reported using immunofluorescent detection or fluorescently-tagged proteins of interest. These techniques have identified key players in DNA repair pathways, and the responses of repair proteins after damage to be quantified. While these techniques have provided detailed information about the kinetics of recruitment or the order of assembly for nucleotide excision repair (NER) and double strand break (DSB) repair [1,2], information is still lacking for single strand break (SSB) and base excision repair (BER) pathways. Also, the global nature of the damaging agent does not allow for a synchronized start to damage initiation or for cellular compartment specific applications.

Fortunately, the past 20 years has seen significant advancement of laser micro-irradiation techniques. Since 1993, when Limoli and Ward demonstrated that UV light can induce strand breaks on bromodeoxyuridine (BrdU)-sensitized DNA [3], a number of groups have leveraged this technique and expanded the use of microlaser technology to examine the recruitment, kinetics, and specificity of DNA repair proteins at sites of laser-induced DNA damage [4–9]. The coupling of laser scanning confocal microscopes and damage-inducing laser wavelengths has created improved tools for inducing base lesions and strand breaks, while allowing the immediate visualization of the repair complex response [4,6,9].

The fields most impacted by the advances in micro-irradiation have been NER and DSB repair [4–7,10,11]. However, a growing number of studies have been examining the response of DNA repair factors to the sites of SSBs and oxidatively-induced base lesions [12–27]. These types of laser-induced DNA damage allow the response of BER proteins to be examined.

BER is primarily responsible for the repair of endogenously or exogenously damaged DNA bases, such as alkylated or oxidized bases. The large number of substrates recognized by BER requires a diverse set of proteins that are responsible for the recognition, removal, and repair of base lesions. A number of DNA glycosylases are involved in damage recognition, as are scaffold proteins, like X-ray repair cross-complementing protein 1 (XRCC1) and poly(ADP-ribose) polymerase 1 (PARP-1), and end-tailoring enzymes like AP-endonuclease (APE1), polynucleotide kinase phosphatase (PNKP) [28,29] and tyrosyl-DNA phosphodiesterase 1 (TDP1). BER is also responsible for repairing SSBs [30], and a number of distinct sub-pathways have been described (e.g., single-nucleotide and long-patch BER) [29–31].

The diversity and complexity of BER make it a rich target for micro-irradiation studies; however, a major limitation to the application of micro-irradiation to the study of BER has been the induction of specific DNA lesions for the study of specific sub-pathways. Recently, a more concerted effort has been focused on examining the dynamics of DNA repair proteins involved in BER. A number of interesting findings have been reported about the recruitment kinetics and persistence of BER repair proteins at sites of DNA damage, and several reports have indicated the possibility of novel sub-pathways that are independent of key scaffold proteins, like XRCC1 or PARP-1 [12,16,19,32].

These studies offer intriguing insights into the complexity of the BER pathway; however, a fundamental limitation to the general acceptance of these results is that the micro-irradiation conditions differ widely in the various reports, and detailed information about the generation and characterization of the induced lesions is often not adequately described. Nevertheless, comparison of a number of published studies reveals some consistencies, despite the use of different techniques to induce DNA damage. This consistency argues that further investigation of BER by micro-irradiation, with improved standardization in reporting of the generation and characterization of damaging conditions, could significantly advance the detailed mechanistic understanding of the BER pathway and its response to SSBs and oxidatively-induced base lesions. A wide variety of techniques have been reported to generate these types of lesions, though only a handful of papers have investigated the behaviors of the BER proteins. To help promote further investigation, we have specifically reviewed the application of