DNA Repair 37 (2016) 33–42

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

The *UVS9* gene of *Chlamydomonas* encodes an XPG homolog with a new conserved domain

Erin Deitsch[a,b], Erin M. Hibbard[b,1], Jason L. Petersen[b,c,d,*]

[a] Center for Biological Control and Analysis by Applied Photonics (BCAAP), South Dakota State University, Brookings, SD, USA
[b] Research and Development, Sioux Falls Veterans Affairs Health Care System, Sioux Falls, SD, USA
[c] Basic Research Division, The Avera Research Institute, Sioux Falls, SD, USA
[d] Department of Internal Medicine, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, USA

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 6 November 2015
Accepted 16 November 2015
Available online 19 November 2015

Keywords:
Nucleotide excision repair
DNA repair
DNA damage
Ultraviolet light
XPG protein
Chlamydomonas reinhardtii

ABSTRACT

Nucleotide excision repair (NER) is a key pathway for removing DNA damage that destabilizes the DNA double helix. During NER a protein complex coordinates to cleave the damaged DNA strand on both sides of the damage. The resulting lesion-containing oligonucleotide is displaced from the DNA and a replacement strand is synthesized using the undamaged strand as template. Ultraviolet (UV) light is known to induce two primary forms of DNA damage, the cyclobutane pyrimidine dimer and the 6-4 photoproduct, both of which destabilize the DNA double helix. The *uvs9* strain of *Chlamydomonas reinhardtii* was isolated based on its sensitivity to UV light and was subsequently shown to have a defect in NER. In this work, the *UVS9* gene was cloned through molecular mapping and shown to encode a homolog of XPG, the structure-specific nuclease responsible for cleaving damaged DNA strands 3′ to sites of damage during NER. 3′ RACE revealed that the *UVS9* transcript is alternatively polyadenylated. The predicted UVS9 protein is nearly twice as long as other XPG homologs, primarily due to an unusually long spacer region. Despite this difference, amino acid sequence alignment of UVS9p with XPG homologs revealed a new conserved domain involved in TFIIH interaction.

Published by Elsevier B.V.

1. Introduction

The genome of cells is exposed to a variety of agents capable of inducing DNA damage. The ultraviolet (UV) component of sunlight is one of these agents and is well-known for inducing the formation of cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs) in DNA [1–3]. These bulky lesions are a threat to cells by stalling DNA replication and transcription, inducing mutation, and potentially causing cell death. To cope with the DNA damage induced by UV light, as well as damage caused by other genotoxic agents, cells have developed several elegant mechanisms of DNA repair [4]. Understandably, cells with defects in these DNA repair processes are often more sensitive than their wild type counterparts to DNA damaging agents, including UV light.

The human genetic disorder xeroderma pigmentosum (XP) is typified by sensitivity to UV light, an increased incidence of skin cancer with sun exposure, and decreased life span. Some cases of XP involve additional traits, including mental retardation and neurological degeneration [5,6]. It is established that the cause of XP is defective nucleotide excision repair (NER), a key pathway for repairing DNA damage [7,8]. NER involves at least 30 proteins [9] and primarily repairs bulky DNA lesions that destabilize the DNA duplex, including CPDs and 6-4 PPs [10]. Seven complementation groups of XP (XP-A through XP-G) result from mutations in NER genes [11].

NER can be divided into several sequential stages, the first of which is DNA damage recognition. More precisely, it is the distortion of the DNA helix caused by DNA damage that is recognized for NER [12]. The proteins involved in damage recognition for NER differ based on whether the damage is located on actively transcribed genes or elsewhere in the genome. In fact, this distinction has led to the division of NER into two subpathways, transcription-coupled NER (TC-NER) and global genome NER (GG-NER). For TC-NER, damage recognition occurs by stalled RNA polymerase II [13,14]. In most cases, damage recognition for GG-NER is performed by a

Abbreviations: UV, ultraviolet; CPD, cyclobutane pyrimidine dimer; 6-4 PP, 6-(1,2)-dihydro-2-oxo-4-pyrimidinyl-5-methyl-2,4-(1H,3H)-pyrimidinedione photoproducts; XP, xeroderma pigmentosum; NER, nucleotide excision repair; TC-NER, transcription-coupled NER; GG-NER, global genome NER; FEN, flap endonuclease; BAC, bacterial artificial chromosome; CRIV, Conserved Region in Viridiplantae; RE, repetitive element.

* Corresponding author at: Sioux Falls VA Healthcare System, Research and Development 151, 2501W. 22nd Street, Sioux Falls, SD 57105, USA. Fax: +1 605 333 6803.
E-mail address: jason.petersen@va.gov (J.L. Petersen).
[1] Present address: Ross School of Business, University of Michigan, Ann Arbor, MI, USA.

http://dx.doi.org/10.1016/j.dnarep.2015.11.003
1568-7864/Published by Elsevier B.V.

heterotrimeric complex consisting of XPC, HHR23B and Centrin2 [15]. The exception is that CPDs are initially recognized by the DNA-Damage Binding (DDB) complex, DDB2/XPE [16–18]. Following damage recognition, both GG-NER and TC-NER use a shared mechanism for the remaining stages of NER. These stages are initiated by the recruitment of the basal transcription factor TFIIH to the damage site where helicase subunits XPD and XPB create a denaturation bubble of about 30 nt surrounding the DNA damage [19]. In the next steps the damaged DNA strand is cleaved on each side of the lesion generating an oligonucleotide containing the DNA damage. This oligonucleotide is removed allowing a new DNA strand to be synthesized using the undamaged strand as template. The nuclease responsible for cleaving 5′ to the site of DNA damage is XPF:ERCC1 [20], while the 3′ nuclease is XPG [21]. The order of cleavage and synthesis has recently been elucidated. Synthesis of the new strand begins following XPF:ERCC1 cleavage, pauses until XPG cleavage has occurred, and then finishes [9,22].

XPG, the 3′ nuclease for NER, is a member of the flap endonuclease (FEN) superfamily of structure-specific nucleases. Some members of the FEN family are involved in RNA metabolism, while others, including XPG, play key roles in maintaining genomic integrity by participating in DNA replication, repair, and/or recombination [23]. The specificity for nucleolytic cleavage by FEN family members is not determined by nucleic acid sequence, but instead by aberrant nucleic acid structures. For instance, XPG cleaves substrates containing ssDNA/dsDNA junctions with 5′ overhangs, which facilitates cleavage 3′ to damage sites during NER [24,25]. Based on amino acid sequence similarity, all members of the FEN superfamily possess N (N-terminal) and I (Internal) domains [26]. Crystal structures from members of this family have shown that the N and I domains physically interact to form the nuclease domain [27,28]. A unique feature for XPG homologs compared to other family members is a large (~600 amino acids) region that separates the N and I domains [29,30]. The function of this, so called, spacer region is still largely unknown. The amino acid sequence of the spacer region is not well conserved between species, though it is acidic in nature. A short region of homology in the N-terminal portion of the spacer region was identified in higher eukaryotes and named Domain 1 (D1) [31]. Subsequently, D1 was shown to play a role in XPG interaction with TFIIH [32].

The unicellular, eukaryotic, green alga *Chlamydomonas reinhardtii* is a useful model organism for characterizing DNA repair pathways [33]. Unlike mammalian cells, which primarily use NER to repair UV-induced DNA damage, *Chlamydomonas* utilizes a blue light-dependent DNA repair process known as photoreactivation as its primary mechanism to reverse DNA damage induced by UV-light [34,35]. Despite this difference, *Chlamydomonas* remains a valuable model for exploring NER. For example, the discovery that the *Chlamydomonas REX1* gene is critical for NER [36] contributed to the discovery that mutation of its human homolog, *GTF2H5*, results in group A trichothiodystrophy [37]. A historic collection of UV-sensitive *Chlamydomonas* strains has been curated, but the molecular basis for the UV-sensitivity found in these strains remains largely unsolved. One of these strains, *uvs9* (*uv*-sensitive 9), was generated by chemical mutagenesis with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (personal communication, G. Small) and subsequently shown to be blocked in the excision of pyrimidine dimers [33,38,39]. Here, we report the molecular basis for the UV-sensitivity of the *Chlamydomonas uvs9* strain.

2. Materials and methods

2.1. Strains

uvs9 was obtained from Dr. Daniel Vlcek (Comenius University, The Slovak Republic) and used to generate the mapping population used in this study. The same strain was crossed with cc-124 mt(−) to generate a *uvs9* mt(−) strain, which has been submitted to the *Chlamydomonas* Resource Center and designated cc-4885. The *uvs9* mt(+) strain used for the remainder of this study was generated by crossing cc-4885 with cc-125 mt(+). This strain has also been submitted to the *Chlamydomonas* Resource Center and designated cc-4884.

2.2. Survival curves

Chlamydomonas strains were logarithmically grown in liquid TAP media [40] and normalized to an $A_{700} = 0.3$. For each normalized culture, a sample was appropriately diluted and plated on TAP agar plates as unirradiated controls. Seven milliliter aliquots of the normalized cultures were UV-irradiated for varying doses in 10-cm petri dishes using a germicidal UV lamp (UVG-54Handheld UV Lamp, UVP). For each dose, the cells were appropriately diluted and plated on TAP agar plates. All plates were placed in the dark for 18 h to prevent photoreactivation and then placed under normal growth conditions for colony growth. Percent survival was calculated by comparing the number of colonies on the UV-treated plates and on the unirradiated control plates.

2.3. Molecular mapping

uvs9 was mated with S1-D2 (cc-2290), a polymorphic field isolate of *Chlamydomonas* [41]. Progeny from this cross were tested for sensitivity to UV light using the following UV spot test. Cells were cultured at 25 °C in TAP medium then replica spotted on TAP plates. One plate served as a no UV growth control. The other plate was irradiated with 150 J/m^2, immediately placed in the dark for 18 h to prevent photoreactivation, and then placed under continuous light from two cool white fluorescent bulbs at a distance of 30 cm (57 μM m^{-2} s^{-1}) for growth. Under these conditions wild type strains form a dense green spot on the test plate after ~4 days, while strains with the *uvs9* mutation do not. Progeny from this cross that were UV-sensitive, and thus had inherited the *uvs9* mutation, were chosen as the mapping population. Genomic DNA was isolated from each member of the mapping population and used as the template for PCRs with primers from the *Chlamydomonas* molecular mapping kit [42]. The primers for a given molecular marker are designed to generate PCR products of different sizes based on whether the marker was inherited from the mutant strain or the polymorphic strain. This provides the means for measuring cosegregation frequency of the molecular marker and the mutation phenotype of the mapping population. The *uvs9* mutation was localized to the left arm of chromosome X using the KAT marker from the molecular mapping kit. The location of the *uvs9* mutation was further refined using primer sequences from a supplemental database of molecular markers not included in the molecular mapping kit. In our mapping population, the *uvs9* mutation showed complete linkage with the following set of primers LGX3a (5′-CCCGCTACCTGCGTCTATTGCCCC-3′), LGX3b (5′-ATGCTTTACGTCTGCTGTGACGTA-3′), and LGX3c (5′-CTGATGCGTTCGTAAAGTACACCG-3′).

2.4. Complementation assays

2.4.1. Cloning UVS9

Bacterial artificial chromosome (BAC) 19D15 [43] was obtained from the Clemson University Genomics Institute. A 14.5 kb HindIII/XhoI fragment from BAC19D15 was subcloned into pBluescript SK+ (Stratagene), creating SK + XPG HX 14.5. This insert spanned ~5.3 kb upstream of *UVS9* into the predicted 12th intron. A 13.3 kb EcoRI/BglII fragment from BAC19D15 was cloned into pSP72 (Promega), creating pSP72 XPG EB13.3. This insert included

Download English Version:

https://daneshyari.com/en/article/1980018

Download Persian Version:

https://daneshyari.com/article/1980018

Daneshyari.com