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Removal of deoxyinosine from the *Escherichia coli* chromosome as studied by oligonucleotide transformation

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ABSTRACT

Deoxyinosine (dI) is produced in DNA by the hydrolytic or nitrosative deamination of deoxyadenosine. It is excised in a repair pathway that is initiated by endonuclease V, the product of the *nfi* gene. The repair was studied in vivo using high-efficiency oligonucleotide transformation mediated by the Beta protein of bacteriophage λ in a mismatch repair-deficient host. *Escherichia coli* was transformed with oligonucleotides containing a selectable A–G base substitution mutation. When the mutagenic dG was replaced by a dI in the oligonucleotide, it lost 93–99% of its transforming ability in an *nfi*$^+$ cell, but it remained fully functional in an *nfi* mutant. Therefore, endonuclease V is responsible for most of the removal of deoxyinosine from DNA. New *nfi* mutants were isolated based on the strong selection provided by their tolerance for transformation by dI-containing DNA. The repair patch for dI was then measured by determining how close to the transforming dG residue a dI could be placed in the oligonucleotide before it interferes with transformation. At the endonuclease V cleavage site, three nucleotides were preferentially removed from the 3′ end and two nucleotides were removed from the 5′ end. dI:dT and dI:dC base pairs gave the same results. Caveats include possible interference by Beta protein and by mispaired bases. Thus, oligonucleotide transformation can be used to determine the relative importance of redundant repair pathways, to isolate new DNA repair mutants, and to determine with high precision the sizes of repair tracts in intact cells.

1. Introduction

Most base lesions in DNA are removed by excision-repair pathways (reviewed in Ref. [1]). The excision may be limited to the damaged nucleotide, but it often extends beyond it. The resulting gaps in the DNA are filled in by a DNA polymerase and sealed by a DNA ligase to produce a repair patch, that is, a tract of one or more newly inserted nucleotides that replace the ones that were excised. Several types of enzymes may participate in the excision steps, which are usually unique to the lesion. Endonucleases may hydrolyze phosphodiester bonds on one or both sides of the lesion. Sometimes, excision is initiated by a glycosylase, which releases the damaged base leaving an abasic site. In that case the C–O bonds on one or both sides of the base-free sugar may be disrupted by an elimination reaction catalyzed by an endolyase activity, which in some cases belongs to the glycosylase itself. The strand break produced by an endonuclease or endolyase provides a site of entry for an exonuclease that may remove the damaged nucleotide or the base-free sugar phosphate. In addition, a

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Abbreviations: oligo(s), oligodeoxyribonucleotide(s); Endo V, endonuclease V; nt, nucleotide; Str^r, streptomycin resistant (or resistance); Str^s, streptomycin sensitive (or sensitivity).

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helicase may be required, either to release a lesion-containing oligodeoxyribonucleotide (oligo) from the DNA or to unwind a lesion-containing end so that it may be degraded by single-strand specific exonucleases.

The size of the repair patch for a given lesion is specific to that lesion and may provide some insight into the repair pathway. A patch length as short as one nucleotide has been reported for the repair of DNA uracil in prokaryotic and eukaryotic systems in vitro [2]. In the VSR (very short patch repair) system of *Escherichia coli*, which removes deaminated 5-methylcytosine from DNA methylation sites, a patch length of between two and ten nucleotides was measured by genetic experiments [3]. The UvrABC system of *E. coli*, which removes bulky adducts, has a patch size of 12 nucleotides in vitro [4]. A single base mismatch in *E. coli* or in mammalian DNA may cause the replacement of as many as 1000 nucleotides [5]. Most patch sizes have been measured in vitro using cell extracts or mixtures of purified enzymes. Working with cell extracts introduces possible artifacts: component proteins may be denatured on isolation, protein complexes may dissociate, repair is measured in the absence of transcription and DNA replication, and effective levels of some enzymes are not reduced by competing lesions as they may be in the cell. Moreover, some system components such as deoxynucleoside triphosphates, metal ions, salts, enzymes and DNA substrates may be added at levels that do not mimic the intracellular conditions. Studies with purified enzymes have many of the same drawbacks and cannot take into account enzymes and accessory proteins that have yet to be discovered.

In only a few cases have measurements been made in vivo. Methods based on the co-conversion of linked genetic markers are usually crude; they can be no more accurate than the frequency of available markers in any particular region. A promising approach examined the removal of mismatches at various distances from lesions in transfected plasmids [6,7]. It may have general applicability, but the physical nature of the plasmid might affect the results.

In this current study, we present a general method for measuring patch sizes in situ in chromosomal DNA. It is precise, unlike other in vivo methods, and it avoids most of the tacit assumptions of the in vitro methods. The technique involves transformation of chromosomal DNA by single-stranded oligos containing a DNA lesion. This type of transformation is quite efficient in *E. coli* that express the Beta protein of bacteriophage λ. This single-stranded binding protein promotes the annealing of oligos to single-stranded chromosomal regions [8] such as those near the replication fork. We can gauge the size of a repair patch by examining the inheritance of a mutagenic base substitution situated at various distances from a DNA lesion in the transforming oligo. The lesion to which this method is applied is deoxyinosine (hypoxanthine deoxyribonucleoside), which arises normally from the hydrolytic [9] or nitrosative [10] deamination of deoxyadenosine in DNA and would lead to an A:T to G:C transition mutation if it were not repaired [11]. Its repair is initiated by endonuclease V (Endo V), which is encoded by the *nfi* gene. The enzyme cleaves the second phosphodiester bond 3′ to the deoxyinosine [12]. The lesion must then be removed by a second DNase cleaving on its 5′ side. This unidentified enzyme could either be an endonuclease or a 3′ → 5′ exonuclease.

The results will be presented in three major parts. In the first, evidence will be presented that Endo V is the major endonuclease that destroys the transforming activity of dI-containing DNA. This will provide the basis of a selection procedure for *nfi* mutants. In the second part, the patch size produced during Endo V-mediated repair will be measured. In the third part, control experiments will demonstrate that in a wild-type cell, dI-containing oligos are not cleaved before they are incorporated into the chromosome; thus, we are examining repair rather than restriction of dI-containing DNA.

2. Materials and methods

2.1. *Bacterial strain construction*

The strains used are listed in Table 1. The *mutS*::Gm insertion in BW1945 and its derivatives is a PCR-mediated replacement [13] of nt 1 to nt 2522 of *mutS* by a gentamycin resistance cassette. The template was plasmid pKD::Gm, in which an 855-bp *Sac*I Gm cassette from plasmid pUCGm [14] replaced a 360-bp *Pvu*II segment of the kanamycin resistance gene in plasmid pKD4 [15]. The primers were ATCACACCCCATTTAATATCAGGGAACCGGACATAACCCCGTGTAGGCTGGAGCTGCTTC and TTACACCAGGCTCTTCAAGCGATAAATCCACTCCAGCGCCCATATGAATATCCTCCTTAG. Disruptions of *mutS*, *nfi*, and λ *exo* genes were verified by PCR. *lacZ* mutations were

Table 1 – *E. coli* strains used[a]

Strain	Description	Source or reference
BW1185	Hfr KL16 *nfi*-1::*cat* thi-1 spoT1 relA1	[46]
BW1892	*mutS*::Gm λcI857 Δ(*cro-bio*) *lacZ* [nt 1384 G → A; ΔGG (nt 1370–71)]	From BW1945 by oligo-mediated gene replacement
BW1904	*mutS*::Gm λcI857 Δ(*cro-bio*) *lacZ* [nt 1384 G → A; ΔGG (nt 1370–71)] *nfi*-1::*cat*	Transduction: P1(BW1185) × BW1892
BW1945	*mutS*::Gm λcI857 Δ(*cro-bio*)	From DY378 by PCR-mediated gene replacement
BW1946	*mutS*::Gm λcI857 Δ(*cro-bio*) *lacZ* [Δ(nt 1370–71)]	From BW1945 by oligo-mediated gene replacement
BW1947	*mutS*::Gm λcI857 Δ(*cro-bio*) *lacZ* (nt 1384 G → A)	From BW1945 by oligo-mediated gene replacement
BW1948	*mutS*::Gm λcI857 Δ(*cro-bio*) *lacZ*(Am) (nt 50 G → A)	From BW1945 by oligo-mediated gene replacement
BW1950	*mutS*::Gm λcI857 Δ(*cro-bio*) Δ(*exo*)::*cat lacZ* [nt 1384 G → A; Δ(nt 1370–71)]	Transduction: P1(HME27) × BW1892
DY378	λcI857 Δ(*cro-bio*)	[13]
HME27	Δ(*argF-lac*)*169 galK* λcI857 Δ(*cro-bio*) Δ(*exo*)::*cat*	[22]

[a] All strains except BW1185 were derivatives of *E. coli* W3110 [*E. coli* K-12 F⁻ λ⁻ IN(*rrnD-rrnE*)1].