RNA editing changes the lesion specificity for the DNA repair enzyme NEIL1

Jongchan Yeo, Rena A. Goodman, Nicole T. Schirle, Sheila S. David, and Peter A. Beal

Department of Chemistry, University of California, One Shields Avenue, Davis, CA 95616

Edited* by Jacqueline Barton, California Institute of Technology, Pasadena, CA, and approved October 8, 2010 (received for review June 27, 2010)

RNA editing reactions modify, insert, or delete nucleotides and can change the coding properties of an RNA molecule (1). Deamination at C6 of adenosine (A) in RNA generates inosine (I) at the corresponding nucleotide position. Because inosine is decoded as guanosine during translation, the introduction of amino acids into a gene product not encoded in the gene (2, 3). Adenosine to inosine editing is widespread in human cells with thousands of transcripts modified, mainly in introns and untranslated regions (4). Current estimates have the number of A to I sites in the human transcriptome at >15,000 with the vast majority of these sites occurring in Alu repeats (5). However, hundreds of A to I sites also occur in nonrepeat sequences with at least 50 different recoding events known in human cells (6, 7). Recoding by adenosine deamination is common in the nervous system with targets including ligand-gated ion channels, voltage-gated ion channels, and G-protein coupled receptors (2, 3, 8). In several of these cases, recoding has a clear effect on the function of the protein. For instance, editing within three different codons in the message for a serotonin receptor changes an intracellular loop that interacts with G proteins and reduces the ability of the receptor to transmit signal into the cell (3). Consistent with these observations, A to I editing is required for nervous system function in metazoans (9–11). However, although both of the enzymes responsible for A to I editing in humans (ADAR1 and ADAR2) are expressed in tissues throughout the body, little is known about the effect of recoding of targets with roles outside the nervous system (7).

A recent whole transcriptome sequence analysis from various human tissues identified over 200 possible A to I editing sites in nonrepeat sequences, including a site predicted to cause recoding in the mRNA for the DNA repair enzyme NEIL1 (lysin 242 AAA codon edited to AIA codon for arginine) (6). NEIL1 plays a key role in the initiation of base excision repair of oxidized base lesions by catalyzing the cleavage of the N-glycosidic linkage to the 2’-deoxyribose (12). This enzyme is capable of removing a wide array of modified DNA bases including thymine glycol (Tg), 5-hydroxycytosine (5-OHC), 5-hydroxycytosine (5-OHU), dihydrothymine (DHT), dihydrouracil (DHU), the formamido-

Fig. 1. Known substrates for the base excision repair glycosylase NEIL1. Abbreviations: 5-OHU, 5-hydroxycytosine; DHU, dihydrouracil; 5-OHC, 5-hydroxycytosine; Tg, thymine glycol; Gh, guanidinohydantoin; DHT, dihydrothymine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Sp, spirotiominodiydantoin; and FapyA, 4,6-diamino-5-formamidopyrimidine.

*This Direct Submission article had a prearranged editor.

† To whom correspondence may be addressed. E-mail: david@chem.ucdavis.edu or beal@chem.ucdavis.edu.

This article contains supporting information online at www.pnas.orglookup/suppl/doi:10.1073/pnas.1009231107//DSSupplemental.
ability to strongly block DNA replication makes it a toxic lesion in cells (17). NEIL1 is also distinct from hOOG1 and NTH1 in catalyzing both β and δ lyase reactions of the abasic site leaving a phosphate group at the 3′ end of the break (18) that allows NEIL1 to participate in an alternative AP-endonuclease independent BER pathway, in addition to short- and long-patch base excision repair (19). Moreover, NEIL1 is also unique among BER glycosylases in its activity with oxidized lesions in single-stranded, bulged and bubble DNA (12, 20). This property of NEIL1 coupled with the established interactions with proteins such as RPA, PCNA, and CSB strongly suggest its involvement in repair during replication and/or transcription (12, 20). The various features of NEIL1 suggest that this glycosylase plays central roles in facilitating repair and initiating different repair pathways dependent on the context and type of lesion encountered.

The three-dimensional structure of human NEIL1 has been solved by X-ray crystallography (21). In addition, while structural data for a complex of human NEIL1 with damaged DNA has not been reported, complexes of related repair glycosylases with DNA containing damaged bases have been structurally characterized (22, 23). From an analysis of these structures, we realized the NEIL1 recoding site is located in the previously identified lesion recognition loop of this family of DNA repair enzymes (Fig. 2) (22). This observation suggested to us that RNA editing might regulate NEIL1 activity by modulating efficiency and/or specificity of damaged base removal. Regulation via RNA editing had not previously been reported for a DNA repair enzyme. Furthermore, such an effect would imply that NEIL1 repair activity is subject to regulation via pathways that control the editing enzyme responsible for recoding.

Results
The Effect of K242R Recoding on NEIL1 Activity. To evaluate the effect of the amino acid change on NEIL1 activity, we overexpressed and purified the protein bearing either lysine (unedited) or arginine (edited) at position 242. We analyzed the effect of the edit on the rate constants for NEIL1 removal of Tg, Gh, and the Sp1 diastereomer from single stranded DNA, duplex DNA, bulge and bubble structure DNA contexts (Fig. 1 and Fig. 3). Under conditions of multiple turnover, NEIL1 exhibits biphasic “burst” kinetics with Sp-containing substrates providing a means to accurately determine the active site fraction (14). Both enzyme forms exhibit similar active fractions indicating that the difference in amino acid at this position does not globally alter protein folding or stability needed for activity. Using the active enzyme concentration, rate constants for the glycosylase step \( k_g \) under single-turnover conditions were measured (Table 1). We found the edited form of NEIL1 cleaves Tg when paired with G in duplex DNA 30 times more slowly than the unedited form, whereas this form reacts nearly three times faster than the unedited form with Gh in the duplex (Table 1). We also observed that when paired with A, the rate constant for Tg removal for the edited form (1.3 ± 0.1 min\(^{-1}\)) is 40-fold reduced from that for unedited NEIL1 (53 ± 8 min\(^{-1}\)). The superior activity of the unedited form for removal of Tg is observed in all contexts including single-strand DNA, bulge and bubble DNA contexts (Table 1). The Gh lesion is more efficiently removed by the edited form in all DNA contexts. The Sp1 diastereomer is a superb substrate for NEIL1 and is efficiently removed by both edited and unedited forms. There are small differences in processing of Sp1 by edited and unedited NEIL1; in duplex contexts, the edited form exhibits a twofold greater activity while in the bubble substrate its activity is twofold reduced. Notably, the editing reaction has altered the NEIL1 repair efficiency in a lesion-specific nature. While the edited form reacts ~150-fold faster with Gh in the duplex substrate than with Tg, this difference is less than twofold for the unedited form. In the bubble DNA context, with both enzyme forms, Gh is preferred over Tg; however, the magnitude of the preference is ~1,500-fold for the edited form and only 25-fold for the unedited form. Clearly, editing modulates the relative lesion specificity of base removal. The basis for the alteration in specificity and glycosylase activity caused by the conservative K to R change in the lesion recognition loop is not obvious based on the structure of the enzyme alone and awaits further detailed structural and mechanistic studies.

The NEIL1 Pre-mRNA Editing Reaction. The recoding site is located in exon 6 near the intron 5/exon 6 boundary in the NEIL1 pre-mRNA. Based on in silico folding, we predicted the edited adenosine resides in an A-C mismatch in a hairpin stem formed by the pairing of the exon to the immediate upstream intronic sequence (Fig. 4) (24). The long duplex and A-C mismatch at the recoding site are features common in ADAR substrates (25). However, it is not possible to predict a priori whether ADAR1 or ADAR2 is responsible for this edit. Identification of the ADAR responsible for the NEIL1 recoding is significant.
because the two ADARs are themselves subject to different regulatory pathways. To determine which ADAR enzyme is responsible, we generated an RNA comprising the 200 nucleotides flanking the recoding site adenosine for in vitro editing assays. This substrate RNA was then subjected to deamination assays with overexpressed and purified human ADAR1 or ADAR2. Importantly, ADAR1 deaminates the central adenosine of the K242 AAA codon more efficiently than does ADAR2 under these conditions (49% vs. 14%) (Fig. 4B). These results support ADAR1 as the editing enzyme primarily responsible for the NEIL1 recoding and implicate ADAR1 regulation in the control of NEIL1 function. In addition to the adenosine at the central position of the codon, we observed editing at the third position as well in these assays. Indeed, this nucleotide is the preferred deamination site for ADAR2 on this substrate. The third nucleotide of the K242 codon is also edited in vivo, but this edit does not cause an amino acid change (6, 26).

To test our secondary structure hypothesis for the RNA editing substrate, we initially introduced mutations that disrupt the predicted duplex near the editing sites by creating changes in nucleotide positions in the intron sequence that replace three pairing interactions (5′-GUC-3′·3′-CGG-5′) with three mismatches (5′-CGG-3′·3′-CGG-5′). Consistent with our prediction, this mutant RNA is not edited by ADAR1 (mutant 1, Fig. 5). This latter result highlights the importance of the A-C mismatch in influencing editing efficiency for ADAR1 at the NEIL1 recoding site. Interestingly, the duplex secondary structure and A-C mismatch at the recoding site appear to be conserved in other vertebrate NEIL1 pre-mRNAs including from mouse, horse, and dog (Fig. S1), suggesting modulation of NEIL1 structure through RNA editing occurs in other species as well.

Changes in NEIL1 Editing in Response to Interferon. The cellular activity of ADAR1 is regulated in a variety of ways (25). To determine if NEIL1 editing is responsive to changes in ADAR1 activity, we treated U87 cells (human glioblastoma) with interferon-α (IFN-α), a condition that stimulates transcription of ADAR1 (27). When we amplify and sequence NEIL1 cDNA from cells with or without prior interferon treatment, we observe recoding only in the treated cells (Fig. 6). Thus, the relative amounts of edited vs. unedited NEIL1 transcripts can be regulated.

<table>
<thead>
<tr>
<th>Context</th>
<th>Unedited</th>
<th>Tg†</th>
<th>Ratio‡</th>
<th>Unedited</th>
<th>Gh†</th>
<th>Ratio</th>
<th>Unedited</th>
<th>Edited</th>
<th>Ratio</th>
<th>Sp1</th>
<th>Edited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex (X:G)</td>
<td>76 ± 10</td>
<td>2.5 ± 0.1</td>
<td>30</td>
<td>130 ± 20</td>
<td>370 ± 40</td>
<td>0.4</td>
<td>120 ± 40</td>
<td>250 ± 20</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single strand</td>
<td>0.6 ± 0.1</td>
<td>0.02 ± 0.01</td>
<td>30</td>
<td>1.2 ± 0.1</td>
<td>2.4 ± 0.6</td>
<td>0.5</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulge</td>
<td>1.4 ± 0.1</td>
<td>0.04 ± 0.02</td>
<td>35</td>
<td>5.0 ± 0.6</td>
<td>13 ± 1</td>
<td>0.4</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bubble</td>
<td>1.2 ± 0.1</td>
<td>0.06 ± 0.02</td>
<td>20</td>
<td>30 ± 6</td>
<td>94 ± 8</td>
<td>0.3</td>
<td>1.3 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rate constants in min⁻¹ measured under single-turnover conditions (20 nM substrate, 200 nM enzyme) at 37°C. Reactions with single-strand DNA did not proceed to completion; slow reactions rates were determined based on initial rate rather than complete fitting of the progress curve.
†Tg pair with G. Rate constants in the same duplex paired with A for edited and unedited NEIL1 are 1.3 ± 0.1 min⁻¹, and 53 ± 8 min⁻¹, respectively. The ratio is 40.
‡Reactions with Gh-containing bulge and bubble substrates did not go to completion (60%–80%); however, similar relative rates for reactions that proceeded to completion were observed at 25°C.

Yeo et al.

PNAS | November 30, 2010 | vol. 107 | no. 48 | 20717
The location of the site of recoding in the proposed lesion recognition loop of NEIL1 is particularly striking. Previous structural work with the bacterial Fpg glycosylase has shown that the corresponding loop becomes ordered in the presence of lesion-containing DNA; however, the manner that FapyG vs. OG lesions are recognized within this loop are completely different (23, 36). These studies illustrate the importance of flexibility in this region to adopt alternative recognition complexes to recognize a wide variety of substrates. Thus, subtle changes in the conformation and structure of this region of NEIL1 may be all that is required to shift the balance in lesion preference from one lesion to another. Interestingly, previous use of protein engineering has also shown that with only one or two mutations the substrate recognition features of some glycosylases can be altered (37). The ability to recognize a wide variety of substrates may be advantageous under normal cellular conditions where the glycosylases are patrolling the genome in search of damage; however, under conditions that produce a particularly mutagenic lesion (such as Gh), different substrate specificity may be beneficial (see below).

The ability of ADAR1 to catalyze deamination in a substrate RNA model of the NEIL1 editing site and the enhanced editing at this site observed in cells treated with IFN-α implicates ADAR1 in NEIL1 recoding. Because ADAR1 is induced in T lymphocytes and macrophages by tumor necrosis factor α (TNF-α) and IFN-γ as well as in various tissues in endotoxin-treated mice, we predict the edited form of NEIL1 would be abundant during inflammation (38). Although the full impact of NEIL1 recoding is not currently known, it is tempting to speculate that the presence of two forms of the NEIL1 enzyme, each with distinct lesion preferences and repair efficiencies, facilitates repair of DNA damage caused by the oxidative burst associated with an inflammatory response. Of note, high levels of oxidative stress have been shown to result in increased expression of NEIL1 and would be expected to favor formation of the hydantoin lesions, which are preferentially repaired by edited NEIL1 (in duplexes) (Table 1) (12). On the other hand, a recent study of lobular breast cancer progression identified ADAR1 as one of the top 5% of genes expressed in the tumor (26). Furthermore, these authors observed nearly quantitative recoding of the NEIL1 message in tumor transcripts. It is possible that prolonged hyper editing of the NEIL1 message leads to changes in the number and types of mutations that accumulate in the genome. Further studies on the consequences of NEIL1 recoding in cells expressing varying levels of ADAR1 are also warranted.

In summary, ADAR1-catalyzed editing of the NEIL1 mRNA causes the genomically encoded AAA lysine codon, corresponding to amino acid position 242 in the lesion recognition loop of the protein, to be converted to a codon for arginine. The two forms of the NEIL1 protein (edited and unedited) have distinctly different enzymatic properties with changes observed for both glycosylase activity and lesion specificity. Editing occurs in a hairpin duplex structure formed near the intron 5/exon 6 boundary in the NEIL1 pre-mRNA. Furthermore, NEIL1 mRNA recoding is regulated extracellularly by interferon, as predicted for an ADAR1-catalyzed reaction. These results suggest a unique regulatory mechanism for DNA repair and extend our understanding of the impact of RNA editing.

**Materials and Methods**

Details for mutagenesis, overexpression and purification of NEIL1, generation of lesion-containing DNA substrates, glycosylase assays, overexpression and purification of ADARs, generation of ADAR substrate RNAs, and deaminase assays can be found in SI Text.

**ACKNOWLEDGMENTS.** We thank Professor Brenda Bass (University of Utah) and Professor Susan Wallace (University of Vermont) for gifts of ADAR1 and NEIL1 expression plasmids, respectively. We also thank Ashok Bhagwat (Wayne State University, P.A.B. and S.S.D.) for helpful discussions. P.A.B. and S.S.D. acknowledge the National Institutes of Health for financial support in the form of Grants R01-GM061115 (P.A.B.) and R01-CA090689 (S.S.D.). R.A.G. was
supported by training Grant T32-GM08799 from National Institute of General Medical Sciences (NIGMS)-NIH. The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the NIGMS or National Cancer Institute (NCI).


