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The unusual UBZ domain of *Saccharomyces cerevisiae* polymerase η

Rachel V. Woodruff[a], Martha G. Bomar[b,1], Sanjay D'Souza[a], Pei Zhou[b], Graham C. Walker[a,*]

[a] *Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
[b] *Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA*

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ABSTRACT

Recent research has revealed the presence of ubiquitin-binding domains in the Y family polymerases. The ubiquitin-binding zinc finger (UBZ) domain of human polymerase η is vital for its regulation, localization, and function. Here, we elucidate structural and functional features of the non-canonical UBZ motif of *Saccharomyces cerevisiae* pol η. Characterization of pol η mutants confirms the importance of the UBZ motif and implies that its function is independent of zinc binding. Intriguingly, we demonstrate that zinc does bind to and affect the structure of the purified UBZ domain, but is not required for its ubiquitin-binding activity. Our finding that this unusual zinc finger is able to interact with ubiquitin even in its apo form adds support to the model that ubiquitin binding is the primary and functionally important activity of the UBZ domain in *S. cerevisiae* polymerase η. Putative ubiquitin-binding domains, primarily UBZs, are identified in the majority of known pol η homologs. We discuss the implications of our observations for zinc finger structure and pol η regulation.

1. Introduction

The genomes of living cells are constantly exposed to a variety of DNA damaging agents that range from endogenously produced reactive metabolic intermediates to exogenous chemical agents and radiation [1]. In spite of cellular DNA repair mechanisms, replication-blocking lesions can persist in the DNA. Replication of such damaged DNA is accomplished by the use of different mechanisms of DNA, such as translesion synthesis (TLS) [2]. TLS, the process in which specialized DNA polymerases directly replicate the damaged DNA, is carried out by multiple non-essential DNA polymerases. Most of them are members of the Y family [3], and many are optimized for the bypass of distinct cognate lesions.

Polymerase (pol) η is a Y family polymerase whose ability to accurately and efficiently bypass UV radiation-induced cyclobutane pyrimidine dimers (CPDs) [4–7] is important for the avoidance of UV-induced skin cancers. Patients lacking a functional pol η suffer from a syndrome known as xeroderma pigmentosum variant (XPV), which is characterized by an increased incidence of cancer, hypermutability, and sensitivity to UV-induced DNA lesions [8,9]. Less deleterious mutations in the *XPV* gene encoding pol η may also predispose patients to melanoma [10]. In addition to UV lesions, pol η is also implicated in the replication of naturally occurring structured regions of DNA [11] and is able to bypass a variety of lesions *in vitro* [12–18]. However, it displays similarly low fidelity (10^{-2} to 10^{-3}) in the replication of both damaged and undamaged DNA templates [19,20].

The catalytic activity of pol η resides in its N-terminal domains, which share sequence homology with the other Y family TLS polymerases [3]. Pol η also includes a Polymerase Associated Domain (PAD), sometimes called the Little Finger, which participates both in DNA binding and in several specific protein–protein interactions [21–24]. Pol η's recruitment to the DNA is mediated by a C-terminal region of 100 to 200 amino acids, which includes a nuclear localization sequence (NLS), PCNA-interacting regions, and a ubiquitin-binding zinc finger domain (UBZ) (Supplementary data Fig. S2A) [25–28].

The UBZ was first recognized as a putative C2H2 zinc finger motif located near the C-terminus of *Saccharomyces cerevisiae* pol η [9,29]. Human pol η contains a similar motif, which was the first UBZ shown to mediate a physical interaction with ubiquitin [30,31]. UBZ motifs have since been identified in several other proteins, including the Y family TLS polymerase κ (κ), human Rad18, and WRNIP1/Mgs1 [25,32,33]. Although its UBZ domain is required for the normal cellular localization of human pol η [27,34,35], the function and significance of the UBZ in pol η remain to be clarified. Some studies report that truncations of human pol η lacking the UBZ sequence are unable to protect cells from DNA damage [27] and are associated with XPV [36], but a more recent study argues that similar truncated forms of human pol η are functional in TLS [37].

* Corresponding author at: Department of Biology, Massachusetts Institute of Technology, 68-633, 77 Massachusetts Ave., Cambridge, MA 02139. USA.
Tel.: +1 617 253 6716; fax: +1 617 253 2643.
E-mail address: gwalker@mit.edu (G.C. Walker).
[1] Present address: Center for Advanced Drug Research (CADRE), SRI International, Harrisonburg, Virginia 22802, USA.

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Table 1
Yeast strains used in this study.

Strain	Genotype	Source
RWY10	*MATα leu2Δ1 his3Δ1 met5Δ0 ura3Δ0 rad5::kanMX rad30::kanMX*	This study
W1588-4C	*MATa leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 RAD5*	Zhao et al. [55]
RWY13	*MATa leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 RAD5 RAD30-TEV-ProA-7His::HIS3MX*	This study
RWY15	*MATa leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 RAD5 rad30::KanMX*	This study
PJ69-4a	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	James et al. [81]

The current model for UBZ function is that the UBZ's interaction with ubiquitin promotes pol η function by increasing the polymerase's affinity for mono-ubiquitinated PCNA [26,38,39], although new evidence points to an additional role for the UBZ which is independent of ubiquitinated PCNA [40]. PCNA ubiquitination at K164 occurs particularly, though not exclusively, in response to DNA damage [41–43], and is required in human cells to increase pol η's residence time in nuclear foci [44]. Genetic studies in yeast show that TLS is dependent on PCNA modification at K164 [42]. PCNA ubiquitination does not increase the catalytic efficiency of the TLS polymerase [45], cause allosteric changes in PCNA structure, or directly interfere with PCNA's interaction with the replicative polymerase [46]. Thus, it is thought that the effect of PCNA ubiquitination on TLS is primarily to increase PCNA's affinity for the TLS polymerase relative to other PCNA-binding factors.

The structure of the UBZ domain from human pol η was determined by NMR to be a classical ββα zinc finger, interacting via the exposed face of its C-terminal α-helix with the canonical hydrophobic patch of ubiquitin [31]. A single zinc ion is coordinated tetrahedrally by the side chains of the two histidines and two cysteines that make up the signature C2H2 motif [31]. In its structure and mode of interaction, the UBZ domain of human pol η is distinctly different from most other ubiquitin-binding zinc fingers, such as the NZF, ZnF-UBP, and RUZ domains [47–51]. Notably, the ubiquitin-binding CCHC-type zinc finger of NEMO displays an architecture and ubiquitin-binding region similar to the human pol η UBZ domain [33]. Both zinc coordination and ubiquitin binding are needed for UBZ function in human pol η, as DNA damage tolerance can be impaired by mutations affecting either zinc-coordinating (C638A and H564A) or ubiquitin-interacting residues (D562A and F655A) within the UBZ domain of human pol η [25,26,44,52].

In *S. cerevisiae* pol η (encoded by *RAD30*), the UBZ can enhance pol η's affinity for ubiquitin–PCNA fusions, as detected by yeast two-hybrid assay [38,53], and can mediate a direct interaction with ubiquitin [54]. However, research into the UBZ's function in pol η is complicated by the presence of an unusual, non-canonical C2H2 zinc finger sequence within the UBZ motif in the *S. cerevisiae* pol η homolog. Whereas the canonical C2H2 zinc finger sequence is **C**xx**C**. . .**H**xxx(x)**H**, the sequence of the UBZ from *S. cerevisiae* polymerase η is **CC**. . .**H**ADY**H**. Although there are two cysteine residues, they are positioned adjacent to one another, such that only one of their side chains is available for zinc coordination. It has thus been unclear whether zinc coordination is required for UBZ function in *S. cerevisiae* pol η.

Here, we have undertaken a study to elucidate the roles of zinc coordination and ubiquitin binding in the function of the UBZ motif of *S. cerevisiae* pol η. We performed a comprehensive alignment 60 putative UBZ motif sequences from 79 unique pol η homologs, and describe the distribution of putative UBZ and UBM sequences in pol η homologs from a broad variety of species. Among all these putative UBZ sequences, the *S. cerevisiae* sequence is unique in lacking a canonical C2H2 zinc finger sequence. Characterization of *S. cerevisiae* pol η mutants confirms the importance of the UBZ motif, and implies that its function is independent of zinc binding but correlates with its ability to bind ubiquitin. We show that zinc binds to and affects the structure of the purified UBZ domain, suggesting that it is a true zinc finger. However, we demonstrate that the UBZ of *S. cerevisiae* pol η is able to interact with ubiquitin even in the absence of a zinc ion.

2. Materials and methods

2.1. Strains and plasmids

The strain used for the experiment shown in Fig. 2B is a BY4741/BY4742 derivative strain constructed by mating of yeast deletion project strains 14255 and 6430. All other UV sensitivity experiments use derivatives of W1588-4C (*MATa leu2-3,112 ade2-1 can1-100 his3-11,15 ura3-1 trp1-1 RAD5*), a W303 strain with wild-type *RAD5* sequence [55]. Deletion of *RAD30* was constructed by gene replacement using PCR-amplified *rad30::KanMX* from the Saccharomyces Genome Deletion Project strain 4255. To produce the TEV-ProA-7His tagged Rad30 fusion protein, the tag cassette was amplified from pYM10 [56] and inserted by homologous recombination to replace the stop codon of *RAD30*. See Table 1 for additional information on strains.

The plasmids pEGUh6 [57] and pEGUh6-RAD30 [58], of which the latter expresses 6His-Rad30 from the GAL10 promoter, were the kind gifts of Zhigang Wang. Roger Woodgate and John McDonald generously provided the plasmid pJM96 (*RAD30* cloned into pRS415), which expresses Rad30 from its native promoter [59]. Mutants were constructed by site-directed mutagenesis using QuikChange, and are listed in Supplementary Table 1.

The construct for production of the human pol η UBZ domain was previously published [31]. A DNA sequence including to the UBZ domain of *S. cerevisiae* polymerase η (encoding amino acid residues 538–609) was cloned (using the primers 5′-CGCGGATCCACTACCAGCTCGAAAGCTG-3′ and 5′-AAACAACAATCTTT TTTCCCCGAAAGAAAG-3′) into the BamH1 and Xho1 sites of the pET28aPB vector (the kind gift of Thomas Schwartz) to produce an N-terminally 6His-tagged yeast UBZ peptide.

PJ69-4A was used for yeast 2-hybrid analysis, transformed with plasmids described previously, which express GBD and GAD fusions of Rad30, Ub*-Pol30*, or Pol30*-Ub* [60]. (The Pol30 protein, product of *POL30*, is the monomeric subunit of the homotrimer PCNA.) In addition, *rad30* mutants for yeast 2-hybrid analysis (both H568L,H572L and C552R,C553R) were constructed by QuikChange mutagenesis (Stratagene) of the *RAD30* plasmids.

2.2. Sequence analyses

Alignments were made using T-Coffee [30] and ClustalW2. BLAST and PSI-BLAST were used to identify homology in the non-redundant protein database (NCBI) [61,62]. Identification of UBZ