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Discovering the nuclear localization signal of Werner Helicase Interacting Protein 1☆

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ABSTRACT

Our study maps the classic nuclear localization signal (cNLS) domain within WRNIP that directs the protein's nuclear positioning.

The contribution of genome instability to human disease is well established. High mutation frequency within the genome is a risk factor for cancer, underlining the significance of investigations into DNA instability's role in the pathogenesis of these diseases. Werner Helicase Interacting Protein 1 (WRNIP1 or WHIP) was initially identified by a yeast 2-hybrid study with DNA RECQ-like helicase, Werner protein (WRN) [1]. WRNIP1 acts as a modulator in DNA repair response, replication fork protection, and even antiviral signaling [1–7]. Like its yeast homolog, Maintenance of Genome Stability (MGS), it shares the exact functional domains: Zinc Finger (UBZ), AAA+ ATPase, and Leucine Zippers (Fig. 1A, WT). Initially, deletion analysis of yeast MGS had shown elevated mitotic recombination rates, which causes genome instability [6]. Loss of Mgs1 in yeast cells results in an increased level of mitotic recombination [6]. Human WRNIP1 is localized to stalled replication forks and cooperates with RAD51 to safeguard these forks. In addition, it is believed to protect the junction point of reversed replication forks from endonucleolytic degradation through binding or shielding [5,7]. Of additional importance are WRNIP1's connections to p53 and the ataxia-telangiectasia mutated (ATM) pathway [4]. DNA damage repair during mitosis is essential since transient inactivation of ATM kinase leaves mitotic cells more sensitive to double-strand breaks (DSBs) [8,9]. Since ATM is a master regulator [10], other critical proteins in DNA damage response must be involved. DSBs are the most harmful genetic lesions whose repair failure may result in genomic instability, tumor formation, or even cell death. Investigations have shown the connection of WRNIP1 and ATM pathway after DNA damage [4]. WRNIP1 responds to replicative stress by prompting ATM signaling and providing genomic stability [4].

WRNIP1's nuclear location is vital for its function, yet its controlling elements are unknown. This letter to BBA will map the classic nuclear localization signal (cNLS) domain within WRNIP1 that directs the protein's nuclear positioning. cNLSs are short peptides or domains that act as a signal that facilitates the transport of proteins from the cytoplasm into the nucleus [11]. These cNLSs can be used as a tag to deliver proteins to the nucleus, as in the case of cNLS-GFP [11]. cNLSs include two groups, termed monopartite and bipartite. Monopartite cNLSs are comprised of a single cluster with a consensus sequence defined as K (K/R) X (K/R), where X is any amino acid [11,12]. Bipartite cNLSs consist of two domains to signal nuclear delivery (Table S1) [11,12]. Bipartite cNLSs are designated by two clusters of 2–3 positively charged amino acids that are separated by a 9–12 amino-acid linker region, and contain several prolines (P) (Table S1). The consensus sequence is R/K(X)$_{10\text{-}12}$KRXK. Markedly, the upstream and downstream clusters of amino acids are interdependent and indispensable and together control the protein localization. Fig. S1 shows the complete amino acid sequence of human WRNIP1 (Q96S55, Uniprot). The amino acid sequence length is 665 with a mass of 72.1 kDa (Fig. S1, panel A) [13]. Upon analysis through several nuclear localization signal predictors, including NLStradamus [11], the stretch of highly basic amino acids from 69 to 74 (KRRRL) of WRNIP1 is predicted as a classic NLS (cNLS). More specifically, it would classified as a monopartite cNLS comprised of a single cluster often found in proteins such as CXC chemokine receptor type 4, cdc6, and oncoprotein c-myc (Table S1) [11,12]. Viruses utilize these short sequences to enter or sneak into the nucleus through the cell's endogenous import mechanisms. Viral proteins like SV40 large T-antigen and CAV viral protein 1, all contain these monopartite cNLSs (Table S1) [11,12].

As shown in Fig. 1, we performed studies to identify WRNIP1's NLS. The top panel of Fig. 1A shows the domains of WRNIP1 and then which domains are deleted in the mutants used in this study. We deleted the ATPase domain in one mutant and deleted amino acid 69–74, the predicted NLS in another Fig. 1A. The mutants were selected for stable expression for these studies. WRNIP1-WT-GFP and mutant proteins-GFPs were stably expressed in HEK293 cells. WRNIP1 is found exclusively in the nucleus (Fig. 1B Top panel, WT-GFP) [13]. We concentrated on deletions of ATPase and NLS domains for our analysis (Fig. 1B, middle and bottom panels) since the leucine zipper and Zn finger domains were previously shown not to affect localization [14,15]. Deletion

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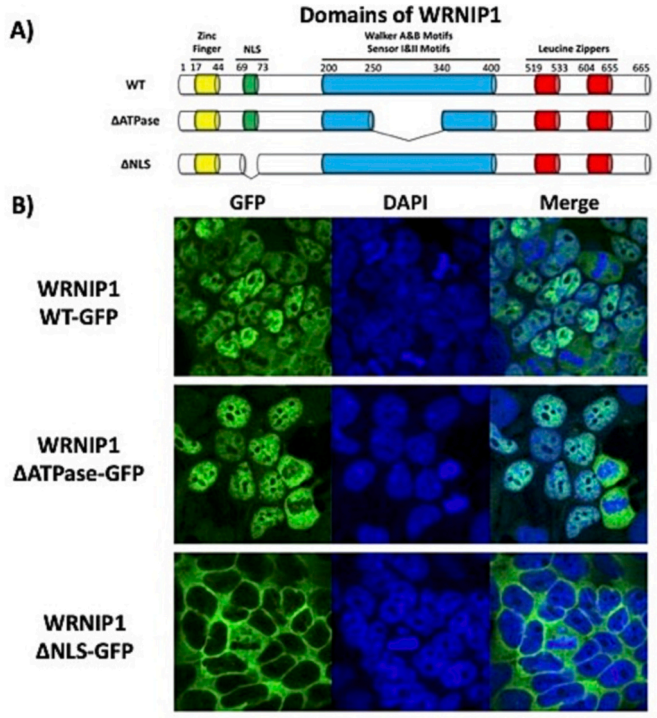

Fig. 1. WRNIP1-GFP expression induced in stably transfected HEK293 cells containing deletions of the ATPase domain (ΔATPase) and cNLS domain (ΔNLS): A) Mapping of WRNIP1's cNLS domain to amino acids 69–74 (KRRRL). Schematic of WRNIP1 protein domains and deletion mutants. B) IF Panels, GFP-tagged WRNIP1-WT-GFP, WRNIP1-ΔATPase-GFP, and WRNIP1-ΔNLS-GFP in HEK293T cells after treating with 1 μM tetracycline for 48 h. The ATPase deletion demonstrates that WRNIP1 can still enter the nucleus. In contrast, the cNLS deletion shows no entry of WRNIP1 into nucleus, indicating that the monopartite cNLS domain alone is critical for intranuclear localization and that the ATPase domain does not regulate it. Methods: To generate these stable expressing cell lines, we used a tetracycline inducible expression system (Tag: His-GFP) as well as Flp-In transfection procedure (Flp-In T-Rex HEK293T, Gateway Cloning Technology, Invitrogen). Our WRNIP1 gene was purchased as a Gateway entry vector from GE life sciences (WRNIP: ORFeome Collab. Hs WRNIP1 ORF w/o Stop Codon, Accession: DQ895136). Deletion mutations were generated using the Quickchange method (Stratagene). Amino acids 69–74 were deleted to generate the ΔNLS-WRNIP. And amino acids 250–340 were deleted to generate the ΔATPase-WRNIP. Cells expressed proteins after treatment with 1 μM/ml tetracycline for 48 h. Nuclei were stained with DAPI. A Zeiss Airyscan 880 confocal microscopy captured images.

of the predicted cNLS, WRNIP1-ΔNLS-GFP exclusively localizes to the cytoplasm (Fig. 1B, bottom IF panel), while WRNIP1-ΔATPase-GFP concentrates in the nucleus (Fig. 1, IF panels). Evidence presented here shows WRNIP1's cNLS domain controls its nuclear localization. In this study, we mapped the cNLS for WRNIP1 through deletion of 69–74 (KRRRL), revealing the change of nuclear localization to the cytoplasm (UniProt) [13]. The role of WRNIP1 is contained in the nucleus and relies on this short sequence of five amino acid residues 69–74 for delivery. Our data indicates that WRNIP's cNLS is biologically functional and therefore essential for WRNIP's function. While sequence analysis of human WRNIP1 predicted this monopatite cNLS, we also find this cNLS conserved in chicken, mouse, rat, Xenopus, and yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) (UniProt) (Fig. S1B) indicating that the domain is functionally conserved over several species.

The cNLS-mediated protein transport delivery mechanism can be divided into three steps: assembly of the cargo-carrier import complex in the cytoplasm, translocation through the Nuclear Pore Complex (NPC), and import-complex disassembly in the nucleus [11,12]. As shown in Fig. S2, WRNIP's delivery to the nucleus would go through this multi-step process starting with recognizing the protein's NLS [11,12]. cNLS on cargo proteins are accepted by the importin α subunit, which binds to the importin β1 subunit [11,12]. cNLS cargo proteins form a trimer with importin α and β1, which depends on the RAN-GTP gradient for transport (Fig. S2) [11,12]. Importin β1 targets importin α to the NPC, then translocates through the NPC via interacting with numerous phenylalanine-glycine (FG) repeats within the pore [11,12]. The nuclear RanGEF (guanine nucleotide exchange factor) leads to the progress of RAN-GTP formation and is the basis of the downstream release of the cargo in the nucleus Fig. S2 [11,12].

Delivery of WRNIP1 to the nucleus is essential for its function. Our results reveal a previously unrecognized monopartite cNLS for WRNIP1. Identification of this small cluster of amino acids gives a new insight for future studies on WRNIP1.

CRediT authorship contribution statement

All authors were involved in the design of the experiments, preparation of figures and references: Benjamin R. Jordan, Yujia Zhai, Zhi Li, Haoshuang Zhao, Marie-Therese Mackmull, and Joseph S. Glavy.

Corresponding author, Joseph S. Glavy prepared the manuscript.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbamcr.2023.119502.

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Benjamin R. Jordan[a], Yujia Zhai[b,c], Zhi Li[b,d], Haoshuang Zhao[b], Marie-Therese Mackmull[d,e,f], Joseph S. Glavy[a,*]

[a] *Department of Pharmaceutical Sciences, Fisch College of Pharmacy, The University of Texas At Tyler, Tyler, TX, USA*
[b] *Stevens Institute of Technology, Hoboken, NJ, USA*
[c] *Memorial Sloan Kettering Cancer Center, NY, NY, USA*
[d] *New York University, Grossman School of Medicine, NY, NY, USA*
[e] *European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany*
[f] *Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, Zurich, Switzerland*

[*] Corresponding author.
E-mail address: jglavy@uttyler.edu (J.S. Glavy).