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Chew, Krystie K.

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MITOCHONDRIAL DNA DAMAGE-BINDING PROTEINS

By

Krystie Chew

A capstone project submitted for Graduation with University Honors

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APPROVED

Dr. Linlin Zhao
Department of Chemistry & Environmental Toxicology

Dr. Richard Cardullo, Howard H Hays Jr. Chair
University Honors

Abstract

Mitochondria have many functions within a eukaryotic cell including cell signaling, programmed cell death, protein cofactor synthesis, and various aspects of metabolism. These organelles carry their own genomic DNA which encodes important proteins involved in oxidative phosphorylation as well as a set of transfer and ribosomal RNAs. Mitochondria are vital for cellular and organismal functions, and damage to mitochondrial DNA (mtDNA) has been linked to many mitochondrial diseases. As such, how the cell maintains the integrity of the mitochondrial genome is an important area of study. Recognizing DNA lesions is a crucial step in repair processes as proteins that bind DNA damage can facilitate or inhibit repair of the damaged DNA. Mitochondrial transcription factor A (TFAM) is a mitochondrial nucleoid protein that is important for the transcription, packaging, and maintenance of mtDNA. Due to its extensive contact with the mitochondrial genome, it is likely to interact with many types of mtDNA damage. This review summarizes the mtDNA damage-binding activity of various proteins with a focus on TFAM. The aim is to discuss how these proteins bind DNA and the role of their DNA damage-binding activity in mtDNA maintenance or immune response.

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1. Introduction

Mitochondria are an important organelle of the cell due to their wide variety of functions including energy production and cell signaling. Mitochondria possess genetic material, mitochondrial DNA (mtDNA). mtDNA is a circular, double-stranded molecule with 16,569 base pairs, which encodes various ribosomal RNAs, transfer RNAs, and important protein subunits involved in oxidative phosphorylation. Even though each mtDNA molecule is much smaller than nuclear DNA in size and multiple copies exist in each mitochondrion, mtDNA has no introns except for a non-coding region. Moreover, mtDNA is more susceptible to damage compared to its nuclear counterpart. Therefore, mtDNA mutations can be detrimental to cell function and human health. For that reason, mitochondria must possess sufficient DNA repair processes to maintain the integrity of mtDNA. However, while nuclei and mitochondria both have base excision repair (BER), mitochondria appear to lack nucleotide excision repair (NER) proteins. This raises the question of how typical NER substrates are handled in the mitochondria. Recently, some proteins involved in nuclear NER have been identified in mammalian mitochondria when the cells were treated with hydrogen peroxide. This might imply that NER proteins typically exist in low or undetectable concentrations in mitochondria but will localize to the organelle under oxidative stress [32]. There is also evidence of noncanonical repair pathways, but the study of these mechanisms is an active research area [32-35].

mtDNA is organized into protein-DNA complexes known as nucleoids. These nucleoids are located in the matrix between the cristae tubules and are separated from the inner boundary membrane by cristae. The mtDNA genome is coated with many nucleoid proteins in which each nucleoid, on average, contains 1-2 mtDNA molecules and thousands of nucleoid proteins [1]. Mitochondrial transcription factor A (TFAM) and mitochondrial single-stranded DNA-binding

protein (mtSSB) are the two most abundant nucleoid proteins. Because nucleoid proteins interact extensively with mtDNA, there is potential for them to encounter various DNA lesions throughout the genome [2]. TFAM, for example, has been noted to bind preferentially to certain types of DNA lesions and subsequently affect repair processes that would normally deal with the damage. As such, interactions between proteins and mtDNA may be important for maintaining mtDNA integrity through recognizing damage or regulating DNA repair. This review aims to summarize the available information about proteins binding to different types of damaged mtDNA.

2. Mitochondrial Transcription Factor A

2.1 Structure and Binding

TFAM is an important DNA-binding protein involved in the packaging, transcription, and maintenance of mtDNA. It is a high-mobility group B protein that is made of two high-mobility group (HMG) box domains, HMG1 and HMG2, separated by a linker region, and a C-terminal tail. The HMG box domains play a principal role in interacting with DNA base pairs in mtDNA. The linker region interacts nonspecifically with the DNA backbone to stabilize the DNA-TFAM interactions, while the C-terminal tail is mainly involved in initiating transcription at the light strand promoter and contributing to strong general binding by TFAM [3,4,30].

TFAM interacts with DNA bases through many of its amino acid residues, but Leucine 58 of HMG1 and Leucine 182 of HMG2 interact specifically with minor groove bases of mtDNA (Figure 1). These residues insert into the minor groove of the DNA molecule, disrupt base-pair stacking, and result in two kinks that impose an overall U-turn structure. HMG1 binds to the light strand promoter (LSP) region of DNA with a greater affinity compared to HMG2.

However, there is a large reduction in DNA bending for both HMG1 and HMG2 alone, indicating that both box domains must be present to induce a U-turn conformation in mtDNA. TFAM imposes this 180° bend on both specific and nonspecific DNA. This effect of TFAM binding is important for both transcription activation and compaction of mtDNA [3,4,5]. Site X and Y are additional binding sites at the control region of mtDNA and have similar sequences to LSP. Binding at these two sites was compared to binding at LSP, and the results indicated that TFAM binding is modulated by sequence-dependent properties. Varying characteristics of the sequences determined the resulting TFAM interactions with DNA as shown by the protein-DNA complex having a stiffer conformation when TFAM is bound to Site X and having a more flexible conformation when TFAM is bound to Site Y. This suggests that TFAM binds to mtDNA in a non-uniform manner and results in uneven distribution of aggregation sites [6]. The mechanism through which TFAM binds DNA can potentially affect the efficiency of lesion recognition and binding throughout mtDNA.

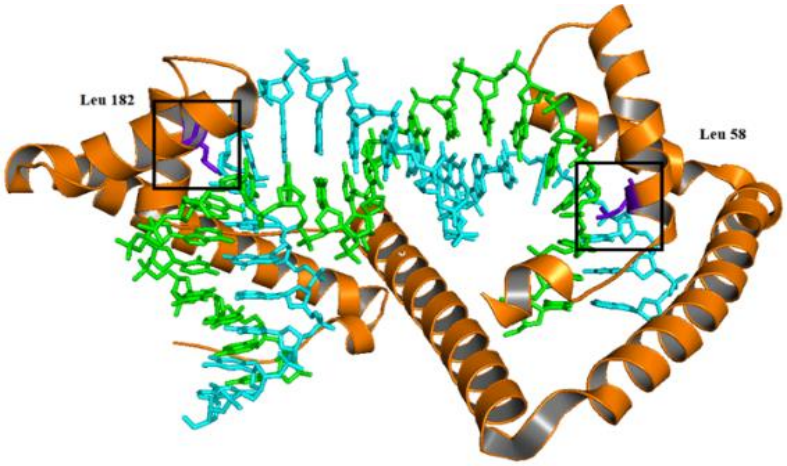


Figure 1. Key residues, Leucine 58 and Leucine 182, identified in purple on TFAM/LSP complex (PBD: 3TQ6).

2.2 Oxidative Damage

TFAM is crucial for packaging DNA and extensively interacts with the mtDNA genome. As such, it is likely to encounter various kinds of DNA lesions that alter DNA. 8-oxo-7,8-dihydroguanine (8-oxo-dG) is an oxidized derivative of deoxyguanosine and is one of the most abundant DNA alterations formed by exposure to reactive oxygen species [7]. 8-oxo-dG is a marker of oxidative damage and is present in mtDNA at levels about three times that of its abundance in nuclear DNA [8]. TFAM is shown to bind more efficiently to 8-oxodG when it is base-paired to either A or C as opposed to when it is base-paired to either T or G. Furthermore, when testing HMG1 and HMG2 binding to 8-oxo-dG alone, HMG1 could bind to damaged DNA while HMG2 could not. This may further support that HMG1 has more significant interactions with DNA base pairs and can more efficiently recognize altered DNA compared to HMG2 [7].

8-oxo-dG is typically repaired via the base excision repair (BER) pathway. TFAM interaction with 8-oxo-dG potentially implicates TFAM as a recognition protein involved in regulating BER. TFAM is shown to reduce the activity of various proteins involved in BER by competitively inhibiting access of these enzymes to DNA. A TFAM mutant was generated by switching Leucine 58, the amino acid that intercalates DNA in HMG1, with Alanine. This mutant has reduced binding to DNA, and it subsequently inhibited BER activity to a lesser degree compared to wild-type TFAM. This evidence suggests that TFAM binding to oxidatively-damaged DNA plays a crucial role in regulating repair pathways. The reason for this activity is unclear, but some have proposed that it may be important for eliminating futile repair cycles, or to accumulate deletions which could contribute to the fast turnover of mtDNA [7,9].

2.3 Abasic Sites

mtDNA degradation is a pathway that is activated when the mitochondria are unable to repair the damage, or if the amount of damage has exceeded the repair capacity. It is nonspecific to lesion type as it occurs with multiple DNA lesions including abasic sites. mtDNA degradation has been shown to be an essential player in maintaining the integrity of mitochondrial genomes [8]. Abasic sites are the most abundant type of endogenous DNA damage in cells. They are very reactive and can result in secondary DNA damage such as DNA single-strand breaks, DNA-DNA interstrand crosslinks, or DNA-protein crosslinks, so it is crucial for the cell to repair these lesions [10]. Unlike for oxidatively-damaged DNA, TFAM shows no significant binding preference for abasic sites under equilibrium conditions [9]. However, TFAM is shown to have a potential role in mitochondrial DNA degradation through facilitating strand cleavage. The stability of abasic sites is reduced upon TFAM binding to the abasic lesion-containing DNA substrates resulting in DNA-TFAM cross-links and DNA strand breaks. Evidence supports that lysine residues in TFAM are responsible for the accelerated strand cleavage as TFAM can form a Schiff base intermediate with the abasic site. Abasic site destabilization was observed in both LSP and nonspecific DNA. The rate of strand cleavage changed with the position of abasic lesion in which AP sites near lysine residues in HMG2 were more susceptible to strand cleavage by TFAM. This implies that lysine residues in HMG2 contribute more to the destabilization reaction. Strand scission that results from this reaction might be a method through which TFAM facilitates mtDNA degradation of damaged mtDNA molecules [10].

2.4 Cytosine methylation of mtDNA

Reversible cytosine methylation is a common form of epigenetic modifications that functions in gene regulation and acts as a hotspot for mutations due to the high rate of

spontaneous deamination of this base to thymine which results in a G/T mismatch [11]. Cytosine methylation could also result from methylating agents from endogenous and environmental sources. CpG sequences are regions of DNA where cytosine is followed by guanine in the 5' to 3' direction. Low levels of cytosine methylation at CpG sequences (5mCpG) have been detected in mitochondrial DNA, but the role of 5mCpG in the mitochondria remains unclear [12]. Considering the important role of TFAM in packaging DNA, TFAM likely encounters many CpG sequences in mitochondrial DNA and modulates CpG recognition and binding. In the nucleus, 5mCpG can modulate the activity of methylation sensitive endonucleases and transcription factors. The proposed mechanism is through direct steric hindrance or direct stabilizing hydrophobic interactions between the major-groove binding proteins and the methyl group. However, TFAM binds to the minor groove rather than the major groove, so the same mechanism cannot be applied as TFAM would not directly interact with the 5-methyl group of cytosine. Methylation of cytosine is not associated with significant changes to B-DNA structure, but DNA methylation can increase the rigidity of DNA through stabilizing base stacking. It is likely that cytosine methylation impacts the relative stability of the TFAM-DNA complex by altering the stacking and deformability of DNA in HSP1. Furthermore, the 5mCpGs in HSP1 are adjacent to the intercalation sites and are about to make further contacts with the C-terminal tail of TFAM. This allows for further interactions that increase base stacking stability and can contribute to the increased affinity of TFAM for 5mCpG DNA. It should be noted that the impact of 5mCpG on TFAM binding does not extend to nonspecific DNA sequences. TFAM does not bind nonspecific sequences in well-defined complexes, and DNA flanking the intercalation sites may hold less importance to nonspecific DNA binding. Since many of the

binding interactions required for DNA packaging are non-sequence specific, it makes sense that 5mCpG has no apparent effect on DNA compaction [12].

2.5 O^4-Alkylthymidine Lesions

Alkylated DNA lesions are a class of DNA damage products that results from exposure to environmental toxicants and/or endogenous metabolites. Alkylating agents can react with nucleobases and the phosphate backbone to generate DNA adducts that can block DNA replication and transcription and induce DNA mutations. Thymidine can be alkylated to generate O^2-alkyldT, O^4-alkyldT, and N3-alkyldT. While N3-alkyldT can be efficiently repaired, O^2-alkyldT and O^4-alkyldT lesions are poorly repaired and tend to persist in mammalian tissues. Several candidate proteins were identified to bind alkylated thymidine lesions, and TFAM, specifically, has been demonstrated to directly and preferentially bind O^4-alkyldT lesions. The binding capability of TFAM to O^4-alkyldT lesions, O^4-nButylthymidine and O^4-pyridyloxobutylthymidine, was measured using electrophoretic mobility shift assay (EMSA). EMSA data provided lower dissociation constants for TFAM binding both types of O^4-alkyldT lesion containing DNA which indicates greater binding affinities toward O^4-alkyldT lesions compared to lesion-free DNA. Given that HMG1 of TFAM plays a predominant role in DNA binding, the binding affinity of each domain alone was also measured. HMG1 showcased higher binding selectivity for O^4-alkyldT DNA compared to full-length TFAM, while HMG2 displayed weak binding to both lesion-free and lesion-containing DNA. As such, the preferential binding of O^4-alkyldT DNA by TFAM is largely a result of the actions of the HMG1 domain. TFAM binding to these lesions was also found to enhance transcriptional bypass. While TFAM overexpression did not alter the efficiency of transcriptional bypass, it did elevate the amount of

transcriptional mutagenesis of the O^4-alkyldT lesions through promoting the misincorporation of guanosine across the lesion site during transcription. However, the mechanism through which TFAM binding modulates transcriptional bypass remains unknown and requires further study [13].

3. Poly [ADP-ribose] polymerase (PARP-1)

Single strand breaks (SSBs) are a common type of DNA lesion characterized as a discontinuity in one strand of DNA. The SSB repair (SSBR) pathway is considered a subset of the short-patch base excision repair pathway as they share the latter steps of the repair process. Poly[ADP-ribose] polymerases (PARPs) are a family of enzymes that can catalyze the transfer of ADP-ribose to target proteins which is important in many cellular processes including DNA repair. In the nucleus, these enzymes are responsible for the detection of SSBs and subsequent initiation of SSBR [14]. PARP-1 binds to single- and double-strand breaks through an N-terminal region containing two zinc fingers, F1 and F2. F2 potentially plays a larger role in damage recognition as it can bind DNA in the same manner whether it is alone or in the two-finger fragment. F2 also binds nicked or gapped DNA ligands with greater affinity compared to F1. [36]. PARP-1 is a predominantly nuclear enzyme that acts as a DNA damage sensor, but it has been shown in some studies to localize to the mitochondria [16]. The role of PARP-1 in mitochondria remains unknown due to conflicting evidence about the effects of PARP-1 depletion on the accumulation and repair of mtDNA damage [15].

Mitofilin is a mitochondrial protein that has been identified as a PARP-1 interactor. Mitofilin associates with outer membrane transport proteins of the mitochondria and likely assists PARP-1 transport into the mitochondria. Evidence shows that knockdown of mitofilin

results in reduced PARP-1 mitochondrial localization and that overexpression of mitofilin appears to increase PARP-1 localization to the mitochondria. PARP-1 has been shown to bind some regulatory and coding sequences of mtDNA which may indicate its role in maintain mtDNA integrity. PARP-1 is shown to interact with DNA ligase III which is an important enzyme in DNA repair as it joins free DNA ends in the BER pathway. In the nucleus, PARP-1 interacts with DNA ligase II during SSBR to regulate its association with mtDNA and to promote mtDNA repair. There is potential for this function of nuclear PARP-1 to have carried over to the mitochondria [16].

To confirm the involvement of PARP-1 in mtDNA maintenance, the amplification of mtDNA was recorded when PARP-1 was knocked down through directly reducing PARP-1 or through depleting mitofilin. Results showed that there was reduced amplification of the mtDNA fragment in both cases, indicating DNA damage accumulation. This implicates PARP-1 as an essential player in mtDNA protection from damage through the promotion of mtDNA repair [16]. However, in other studies, PARP-1 is found to result in a negative effect on mtDNA repair. PARP-1 was shown to interact with key enzymes of the BER pathway, but the repair of oxidative-induced damage to mtDNA was reduced for control cells compared to PARP-1-depleted cells [17]. Furthermore, PARP-1 has also been shown to regulate the repair activity of mitochondrial DNA polymerase (Pol γ) in a NAD+-dependent manner. At low NAD+ levels, PARP-1 inhibits Pol γ but increasing NAD+ allows Pol γ to resume repair activity [18]. As such, the exact role and mechanism of PARP-1 in mtDNA repair remains unclear and further studies are required to determine the effect of PARP-1 binding to mtDNA damage on repair.

4. Y-box binding protein (YB-1)

Nuclear mismatch repair (MMR) is initiated after the recognition of DNA mispairs by MSH proteins. These proteins bind the mismatched DNA and recruit MLH proteins to the damage site and activate downstream molecules to repair the DNA. However, MMR in the mitochondria occurs independently of these proteins and relies on a protein known as the Y-box binding protein (YB-1) [15, 19]. Cells lacking some MSH proteins still showed mitochondrial MMR activity supporting that the mitochondrial MMR pathway is distinct from nuclear MMR. Moreover, nuclear mismatch-binding proteins do not localize to the mitochondria. YB-1, on the other hand, was found to localize to the mitochondria [19]. YB-1 is a multifunctional protein involved in important processes including the regulation of transcription and translation. There is also evidence that it interacts with various DNA repair proteins and is potentially involved in regulating nuclear BER and MMR pathways [20, 21]. Binding specificity of recombinant YB-1 was observed using electrophoretic mobility shift assays (EMSA) and YB-1 was found to bind specifically to mismatched substrate *in vitro*. To assess if YB-1 contributes to DNA mismatch binding *in vivo*, binding to mismatched DNA was observed in YB-1-deficient cells. The loss of YB-1 was accompanied by reductions in mismatched DNA binding, suggesting that YB-1 does play a role in binding mismatched DNA in mitochondria. YB-1 involvement in MMR is also shown by comparing MMR activity in control cells to MMR activity in YB-1-deficient cells. There was decreased mismatch repair in YB-1-deficient cells which correlates with the decreased mismatch binding activity as mentioned before. Addition of recombinant YB-1 to the deficient cells restored MMR activity to normal levels implicating YB-1 in the MMR pathway. To differentiate nuclear MMR contribution from the recorded activities, similar assays were performed with the addition of aphidicolin which inhibits nuclear DNA polymerases α and δ, enzymes required for nuclear MMR activity. Aphidicolin does not inhibit mitochondrial Pol γ, so

mitochondrial MMR activity remains unaffected. These additional assays further suggest that the nuclear MMR pathway is distinct from the mitochondrial MMR pathway [19]. Moreover, there is evidence that YB-1 acts as a negative regulator of MMR in nucleus unlike in mitochondria which require YB-1 for successful MMR [21]. As such, while there is evidence of YB-1 involvement in mitochondrial MMR, its molecular mechanism through which it interacts with the mitochondrial MMR system requires further study.

5. Nucleotide-binding domain leucine-rich repeat family, pyrine domain containing 3 (NLRP3)

Mitochondria protein binding damaged DNA also plays an important role in the immune response. 8-oxo-dG is a common indicator of oxidative damage, and there is evidence of oxidatively-damaged mtDNA being involved in triggering an immune defense program. Nucleotide-binding domain leucine-rich repeat family, pyrine domain containing 3 (NLRP3) is a component of the innate immune system and functions as a pattern recognition receptor in the cytosol where it recognizes molecules found in pathogens or molecules released from damaged cells. NLRP3 forms an inflammasome complex with the adaptor ASC protein to activate capase-1. NLRP3 inflammasome activation has been shown to be linked to apoptosis and mitochondrial activity which led to further study on the mechanism through which they interact [22-24]. In multiple studies, it was found that both mitochondria-derived reactive oxygen species (ROS) and mtDNA release are required for NLRP3 activation [22, 23]. While one group originally concluded that NLRP3 is required for mtDNA release because mtDNA was not detected in NLRP3-deficient macrophages, later evidence showed that NLRP3 can bind oxidized DNA regardless of its source [22, 27]. NLRP3 activators trigger new mtDNA synthesis to generate

oxidized mtDNA fragments that will be released via membrane pores that can drive NLRP3 inflammasome activation, or the inflammasome can be activated by mtDNA released during apoptosis [24-27]. This indicates that NLRP3 itself does not trigger mtDNA release, and that the protein likely binds to mtDNA released into the cytosol and subsequently stabilizing it [22, 24]. As such, oxidized mtDNA directly binding to NLRP3 is required to activate the NLRP3 inflammasome [24, 27]. Moreover, oxidized dG inhibits NLRP3 activation by preventing binding of oxidized mtDNA further supporting that oxidized mtDNA binding is required for NLRP3 inflammasome activation [24]. While this data supports that binding is necessary for activation, the mechanism of NLRP3 binds oxidized mtDNA is unknown and an evaluation of binding with different forms of oxidized DNA lesions is needed.

6. Conclusions

The ability of proteins to bind damaged DNA is important for maintaining cellular and organismal health. TFAM is a large player in protecting mtDNA integrity through its ability to recognize and bind damaged DNA. Its interactions with various types of DNA lesions facilitate repair through either destabilizing the DNA lesion, or through interacting with repair proteins. TFAM binding to DNA damage can have stimulatory or inhibitory effects on different repair pathways. For example, TFAM binding oxidatively-damaged mtDNA appears to inhibit BER [7]. Because mtDNA degradation has been demonstrated as an essential mechanism in mtDNA maintenance, inhibition of this repair pathway by TFAM may be a means of accumulating mtDNA to trigger faster mtDNA turnover [2]. TFAM binding to abasic or O^4-alkyldT containing DNA may also promote mtDNA degradation by facilitating the accumulation of damage. In the case of abasic sites, TFAM binding results in destabilization of the DNA resulting in strand

cleavage and the formation of strand breaks [10]. TFAM binding of O^4-alkyldT lesions promote mutagenic transcriptional bypass due to the misincorporation of guanosine across these lesions [13]. In these cases, TFAM binding to the lesions result in the formation or preservation of damage in mtDNA. While that might sound detrimental to cellular health, each mitochondrion contains multiple variants of the mitochondrial genome. This allows mtDNA degradation to be a feasible pathway for dealing with damage in the mitochondria. mtDNA is known to be more susceptible to damage and has a higher turnover rate compared to nuclear DNA [2]. This may be attributed to mtDNA degradation being a prevalent mechanism for dealing with DNA damage as these proteins may contribute to initiating degradation. Furthermore, there is evidence of increased binding of TFAM to damaged hot spots in aged rat heart potentially being linked to mtDNA loss which might further support that TFAM binding regulates repair processes by hindering normal repair cycles [31]. Taken together, this may further implicate TFAM in mtDNA degradation as well as support the prevalence of degradation in maintenance.

TFAM binding DNA has sequence-dependent characteristics due to the varying conformations of DNA sequences [6]. DNA damage is often able to induce changes in DNA structure which can affect how proteins recognize and bind to the DNA. The sequence-dependent binding characteristic of TFAM can have implications on how binding to mtDNA lesions might change depending on its location in the genome. The sequence surrounding a DNA lesion may have a certain conformation that can be further distorted by the damage. This might suggest that the binding activity of TFAM to a certain type of lesion can vary between sequences that the damage is found in. As such, it might be useful to observe how the binding ability of TFAM might change between varying sequences and discern how this might affect damage recognition and repair processes. The non-uniform binding by TFAM also suggests that the aggregation of

TFAM on DNA due to packaging is uneven. This might imply variability in the efficiency of damage recognition and subsequent repair dependent on the location of the damage. Assuming different parts of the mitochondrial genome are more saturated with TFAM compared to others, areas with less aggregation might experience greater damage as well as damage recognition by other proteins with damage-binding capabilities as the area is more accessible for them to bind. Therefore, it may be beneficial to observe TFAM binding to DNA damage in the context of sequence and location.

The activity of TFAM in regulating repair processes through DNA damage binding shows potential for other proteins to carry out similar actions. There are many other mitochondrial nucleoid proteins that were not discussed in this review. While many of them are implicated in mtDNA maintenance and some also interact with damaged mtDNA, there was a lack of reports about the binding activity of these proteins to damaged mtDNA. This area may be worth studying because nucleoid proteins make extensive contact with mtDNA, similar to TFAM, providing many opportunities for them to interact with damaged mtDNA. Lon protease (Lon), for example, is important for the degradation of DNA-bound proteins. Lon is known to bind DNA nonspecifically and can bind preferentially to DNA sequences that are likely to form G-quadruplex structures [28]. This indicates that Lon can interact differently with varying sequences of DNA. While DNA binding is known to modulate Lon activity, the mechanism of this remains unknown. However, based on this information, there is potential that Lon is able to recognize specific DNA modifications or damage which, in turn, can trigger a specific response in Lon activity. Other nucleoid proteins have been shown to interact with damaged mtDNA, but the effect on enzymatic activity is more often reported. Observing how these protein bind to damaged-DNA may give light to new mechanisms in mtDNA repair or maintenance.

While mitochondria and nuclei both have different repair pathways for maintaining their respective genomes, there are some points of overlap. PARP-1 is a protein involved in nuclear DNA repair of single strand breaks. While this protein binds and facilitates repair in the nucleus, its role in mitochondrial SSB repair still requires further study. In the case of MMR, the main binding protein in the mitochondria is YB-1 while MLH proteins recognize mismatched DNA in the nucleus. Furthermore, YB-1 appears to have opposite effects on MMR in nuclei and in mitochondria. Both proteins are based in the nucleus but can translocate to the mitochondria in the event of mitochondria stress. Crosstalk between the nucleus and mitochondria is crucial for mtDNA maintenance because many proteins involved are nuclear DNA-encoded. This implicates not only mitochondrial nucleoid proteins, which come into contact with mtDNA quite frequently, but also nuclear protein that may localize to mitochondria in maintaining mtDNA through binding DNA lesions.

In this review, the damage-binding protein, NLRP3, which is involved in immune response, was also discussed. As aforementioned, oxidized mtDNA binds to this protein to activate the NLRP3 inflammasome complex. In addition, there is also evidence of oxidized mtDNA triggering inflammation through binding Z-DNA binding protein 1 (ZBP-1) [29]. This interaction is not as well supported, but both these proteins indicate other potential interactions between damaged mtDNA and immune response proteins. Mitochondria have many roles in immune response, and damaged mtDNA may play a large role in triggering different responses. NLRP3 activators are able to initiate new mtDNA synthesis to generate the necessary oxidized mtDNA to activate the inflammasome complex [27]. This implicates oxidized mtDNA as a specific signaling molecule for the immune response. Potentially, other types of damaged DNA could be generated from the mitochondria to signal different types of immune proteins, or

oxidized mtDNA may be able to bind a large variety of different proteins that have not yet been identified. Elucidating the pathways of immune response is important for understanding the mechanisms through which our bodies protect itself and understanding the potential consequences if any points in these processes were compromised.

mtDNA damage-binding proteins are an important topic to study because mtDNA damage has been implicated in many mitochondrial diseases. Not only is it important to identify which proteins can recognize DNA damage to initiate repair or immune response, it is also valuable to elucidate the binding mechanisms of these proteins. There is a lot of evidence that supports the mtDNA-damage binding activity of these proteins, but there is less in-depth information about the molecular binding mechanism. Binding information would also be useful to better understand how these proteins can recognize and interact with damaged DNA, and to better comprehend how binding might affect the conformation of the proteins and allow for the subsequent interactions that initiate the intended biological response. All in all, this information can help provide insight for the development of therapeutics for mitochondrial disease.

References

1. Kukat, C., Davies, K. M., Wurm, C. A., Spåhr, H., Bonekamp, N. A., Kühl, I., Joos, F., Polosa, P. L., Park, C. B., Posse, V., Falkenberg, M., Jakobs, S., Kühlbrandt, W., & Larsson, N.-G. (2015). Cross-strand binding of TFAM to a single mtDNA molecule forms the mitochondrial nucleoid. *Proceedings of the National Academy of Sciences*, *112*(36), 11288–11293.
2. Zhao, L. (2019). Mitochondrial DNA degradation: A quality control measure for mitochondrial genome maintenance and stress response. In *The Enzymes* (Vol. 45, pp. 311–341).
3. Rubio-Cosials, A., Sidow, J. F., Jiménez-Menéndez, N., Fernández-Millán, P., Montoya, J., Jacobs, H. T., Coll, M., Bernadó, P., & Solà, M. (2011). Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. *Molecular Biology*, *18*(11), 10.
4. Ngo, H. B., Kaiser, J. T., & Chan, D. C. (2011). The mitochondrial transcription and packaging factor Tfam imposes a U-turn on mitochondrial DNA. *Nature Structural & Molecular Biology*, *18*(11), 1290–1296.
5. Ngo, H. B., Lovely, G. A., Phillips, R., & Chan, D. C. (2014). Distinct structural features of TFAM drive mitochondrial DNA packaging versus transcriptional activation. Nature Communications, 5(1), 3077.
6. Cuppari, A., Fernández-Millán, P., Battistini, F., Tarrés-Solé, A., Lyonnais, S., Iruela, G., Ruiz-López, E., Enciso, Y., Rubio-Cosials, A., Prohens, R., Pons, M., Alfonso, C., Tóth, K., Rivas, G., Orozco, M., & Solà, M. (2019). DNA specificities modulate the binding of human transcription factor A to mitochondrial DNA control region. Nucleic Acids Research, 47(12), 6519–6537.
7. Yoshida, Y., Izumi, H., Ise, T., Uramoto, H., Torigoe, T., Ishiguchi, H., Murakami, T., Tanabe, M., Nakayama, Y., Itoh, H., Kasai, H., & Kohno, K. (2002). Human mitochondrial transcription factor A binds preferentially to oxidatively damaged DNA. Biochemical and Biophysical Research Communications, 7.
8. Zhao, L., & Sumberaz, P. (2020). Mitochondrial DNA Damage: Prevalence, Biological Consequence, and Emerging Pathways. Chemical Research in Toxicology, 33(10), 2491–2502.
9. Canugovi, C., Maynard, S., Bayne, A.-C. V., Sykora, P., Tian, J., de Souza-Pinto, N. C., Croteau, D. L., & Bohr, V. A. (2010). The mitochondrial transcription factor A functions in mitochondrial base excision repair. DNA Repair, 9(10), 1080–1089.
10. Xu, W., Boyd, R. M., Tree, M. O., Samkari, F., & Zhao, L. (2019). Mitochondrial transcription factor A promotes DNA strand cleavage at abasic sites. Proceedings of the National Academy of Sciences, 116(36), 17792–17799.
11. Walsh CP, Xu GL. Cytosine methylation and DNA repair. Curr Top Microbiol Immunol. 2006; 301:283-315.

12. Dostal, V., & Churchill, M. E. A. (2019). Cytosine methylation of mitochondrial DNA at CpG sequences impacts transcription factor A DNA binding and transcription. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1862(5), 598–607.
13. He, X., Wang, P., & Wang, Y. (n.d.). Mitochondrial Transcription Factor A Binds to and Promotes Mutagenic Transcriptional Bypass of O4-Alkylthymidine Lesions. Analytical Chemistry, 11.
14. Morales, J., Li, L., Fattah, F. J., Dong, Y., Bey, E. A., Patel, M., Gao, J., & Boothman, D. A. (2014). Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Critical reviews in eukaryotic gene expression, 24(1), 15–28.
15. Saki, M., & Prakash, A. (2017). DNA damage related crosstalk between the nucleus and mitochondria. Free radical biology & medicine, 107, 216–227.
16. Rossi M.N., Carbone M., Mostocotto C., Mancone C., Tripodi M., Maione R., Amati P. Mitochondrial localization of PARP-1 requires interaction with mitofilin and is involved in the maintenance of mitochondrial DNA integrity. J. Biol. Chem. 2009;284:31616–31624.
17. Szczesny, B., Brunyanszki, A., Olah, G., Mitra, S., & Szabo, C. (2014). Opposing roles of mitochondrial and nuclear PARP1 in the regulation of mitochondrial and nuclear DNA integrity: implications for the regulation of mitochondrial function. Nucleic acids research, 42(21), 13161–13173.
18. Herrmann GK, Russell WK, Garg NJ, Yin YW. Poly(ADP-Ribose) polymerase 1 regulates mitochondrial DNA repair in a metabolism-dependent manner. J Biol Chem. 2021 Jan 19;296:100309.
19. de Souza-Pinto, N. C., Mason, P. A., Hashiguchi, K., Weissman, L., Tian, J., Guay, D., Lebel, M., Stevnsner, T. V., Rasmussen, L. J., & Bohr, V. A. (2009). Novel DNA mismatch-repair activity involving YB-1 in human mitochondria. DNA repair, 8(6), 704–719.
20. Gaudreault, I., Guay, D., & Lebel, M. (2004). YB-1 promotes strand separation in vitro of duplex DNA containing either mispaired bases or cisplatin modifications, exhibits endonucleolytic activities and binds several DNA repair proteins. Nucleic acids research, 32(1), 316–327.
21. Chang, Y. W., Mai, R. T., Fang, W. H., Lin, C. C., Chiu, C. C., & Wu Lee, Y. H. (2014). YB-1 disrupts mismatch repair complex formation, interferes with MutSα recruitment on mismatch and inhibits mismatch repair through interacting with PCNA. Oncogene, 33(43), 5065–5077.
22. Nakahira, K., Haspel, J. A., Rathinam, V. A., Lee, S. J., Dolinay, T., Lam, H. C., Englert, J. A., Rabinovitch, M., Cernadas, M., Kim, H. P., Fitzgerald, K. A., Ryter, S. W., & Choi, A. M. (2011). Autophagy proteins regulate innate immune responses by inhibiting the

release of mitochondrial DNA mediated by the NALP3 inflammasome. Nature immunology, 12(3), 222–230.
23. Zhou, R., Yazdi, A. S., Menu, P., & Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. Nature, 469(7329), 221–225.
24. Shimada, K., Crother, T. R., Karlin, J., Dagvadorj, J., Chiba, N., Chen, S., Ramanujan, V. K., Wolf, A. J., Vergnes, L., Ojcius, D. M., Rentsendorj, A., Vargas, M., Guerrero, C., Wang, Y., Fitzgerald, K. A., Underhill, D. M., Town, T., and Arditi, M. (2012) Oxidized Mitochondrial DNA Activates the NLRP3 Inflammasome during Apoptosis. Immunity 36, 401−414.
25. Riley, J. S., Quarato, G., Cloix, C., Lopez, J., O'Prey, J., Pearson, M., Chapman, J., Sesaki, H., Carlin, L. M., Passos, J. F., Wheeler, A. P., Oberst, A., Ryan, K. M., & Tait, S. W. (2018). Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis. The EMBO journal, 37(17), e99238.
26. Esteve, J. M., Mompo, J., Garcia de la Asuncion, J., Sastre, J., Asensi, M., Boix, J., Vina, J. R., Vina, J., & Pallardo, F. V. (1999). Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies in vivo and in vitro. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 13(9), 1055–1064.
27. Zhong, Z., Liang, S., Sanchez-Lopez, E., He, F., Shalapour, S., Lin, X. J., Wong, J., Ding, S., Seki, E., Schnabl, B., Hevener, A. L., Greenberg, H. B., Kisseleva, T., & Karin, M. (2018). New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature, 560(7717), 198–203.
28. Zeinert, R., Liu, J., Yang, Q., Du, Y., Haynes, C., & Chien, P. (2018). A legacy role for DNA binding of lon protects against Genotoxic stress. bioRxiv, 317677.
29. Szczesny, B., Marcatti, M., Ahmad, A. et al. (2018) Mitochondrial DNA damage and subsequent activation of Z-DNA binding protein 1 links oxidative stress to inflammation in epithelial cells. Sci Rep 8, 914.
30. Ohgaki K, Kanki T, Fukuoh A, Kurisaki H, Aoki Y, Ikeuchi M, Kim SH, Hamasaki N, Kang D. The C-terminal tail of mitochondrial transcription factor a markedly strengthens its general binding to DNA. J Biochem. 2007 Feb;141(2):201-11.
31. Chimienti, G., Picca, A., Sirago, G., Fracasso, F., Calvani, R., Bernabei, R., Russo, F., Carter, C. S., Leeuwenburgh, C., Pesce, V., Marzetti, E., & Lezza, A. M. S. (2018). Increased TFAM binding to mtDNA damage hot spots is associated with mtDNA loss in aged rat heart. Free Radical Biology and Medicine, 124, 447–453.
32. Zinovkina, L. A. (2018). Mechanisms of Mitochondrial DNA Repair in Mammals. Biochemistry (Moscow), 83(3), 233–249.
33. Alencar, R. R., Batalha, C., Freire, T. S., and de Souza-Pinto, N. C. (2019) Enzymology of mitochondrial DNA repair. Enzymes 45, 257−287.
34. Stein, A., and Sia, E. A. (2017) Mitochondrial DNA repair and damage tolerance. Front. Biosci., Landmark Ed. 22, 920−943

35. Bess, A. S., Crocker, T. L., Ryde, I. T., & Meyer, J. N. (2012). Mitochondrial dynamics and autophagy aid in removal of persistent mitochondrial DNA damage in Caenorhabditis elegans. Nucleic Acids Research, 40(16), 7916–7931.
36. Eustermann, S., Videler, H., Yang, J. C., Cole, P. T., Gruszka, D., Veprintsev, D., & Neuhaus, D. (2011). The DNA-binding domain of human PARP-1 interacts with DNA single-strand breaks as a monomer through its second zinc finger. Journal of molecular biology, 407(1), 149–170.