Decoding Nucleic Acid Signals of Environmental Chemical Exposures

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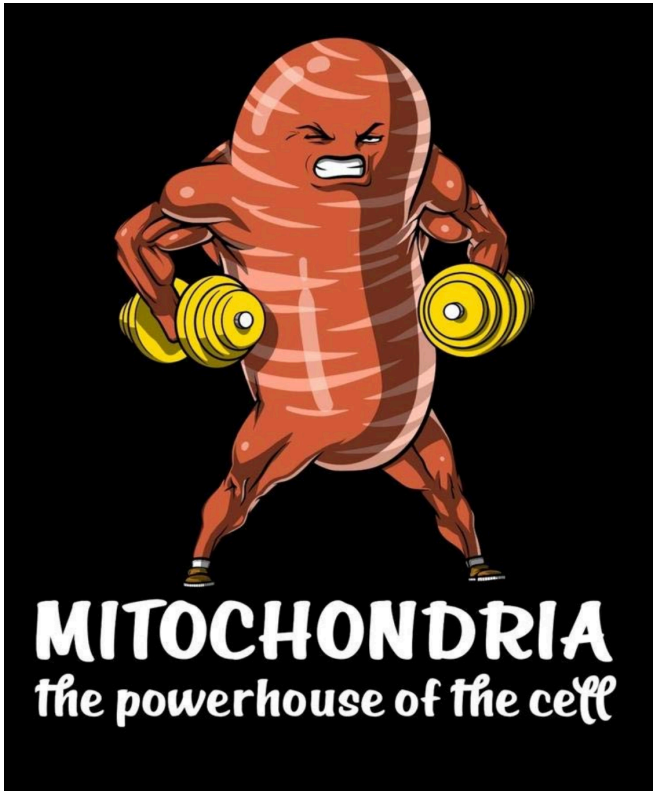
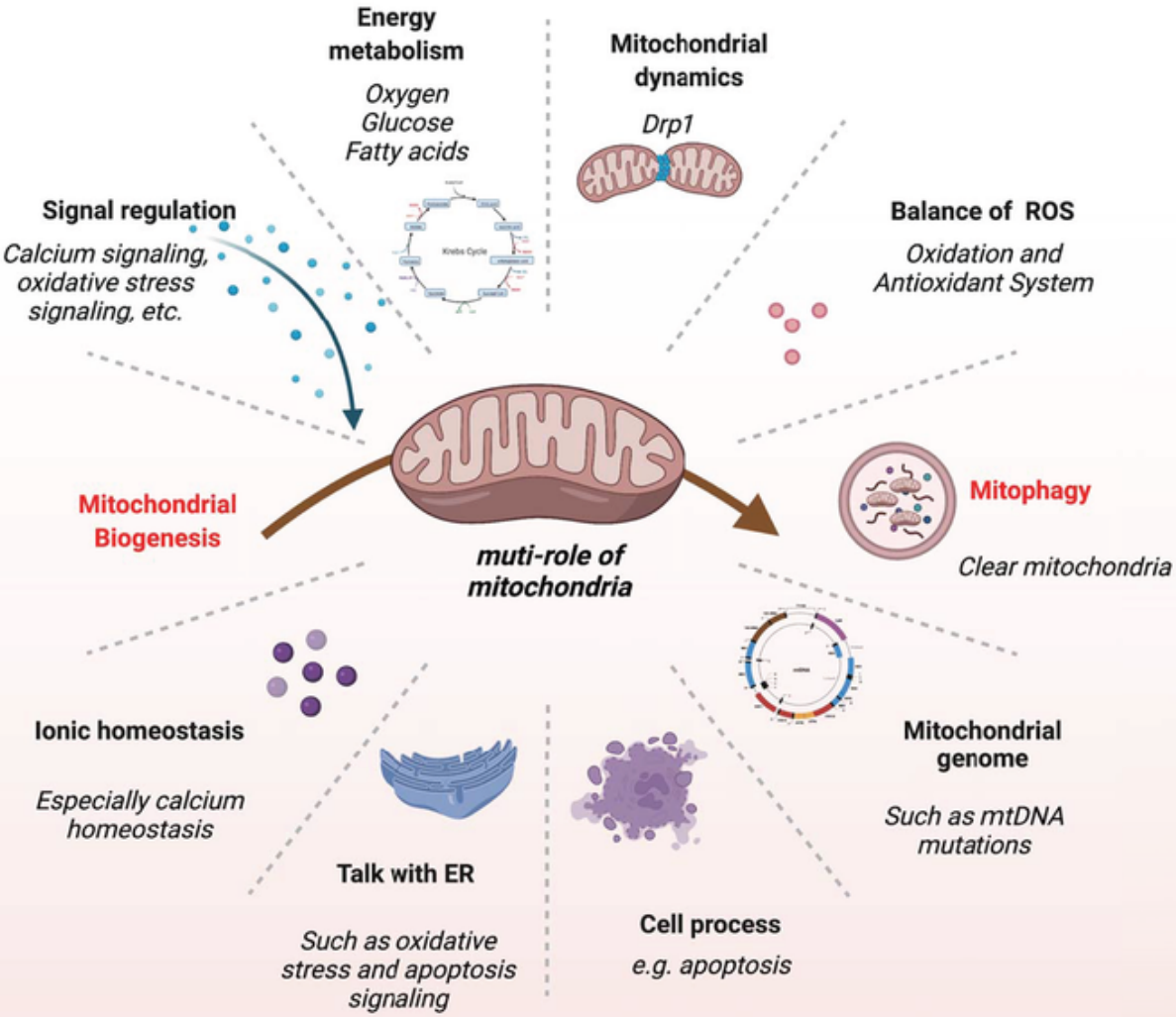
Mitochondria – More Than the Powerhouse



Source - Mitochondria The Powerhouse Of The Cell Science Notebook: Funny Biology Notebook

Mitochondria – More Than the Powerhouse



Zeng et al. Front. Cardiovasc. Med. 2022

Mitochondrial Diseases

Mitochondrial diseases affect parts of your body that need the most energy – heart, brain, muscles – are most affected by mitochondrial disease. An affected individual may exhibit a spectrum of symptoms.

Brain

developmental delays, dementia, migraines, autistic features, seizure, stroke, atypical cerebral palsy, learning disabilities

Muscles

weakness/failure, cramping, reflux, vomiting, constipation, diarrhea, hypotonia, dysmotility

Nerves

fainting, zero reflexes, heat/cold intolerance, pain

Pancreas

diabetes, pancreatic failure, parathyroid failure

Kidneys

renal tube failure

Heart

defects, blockage, cardiomyopathy

Liver

low blood sugar, liver failure

Ears

hearing loss

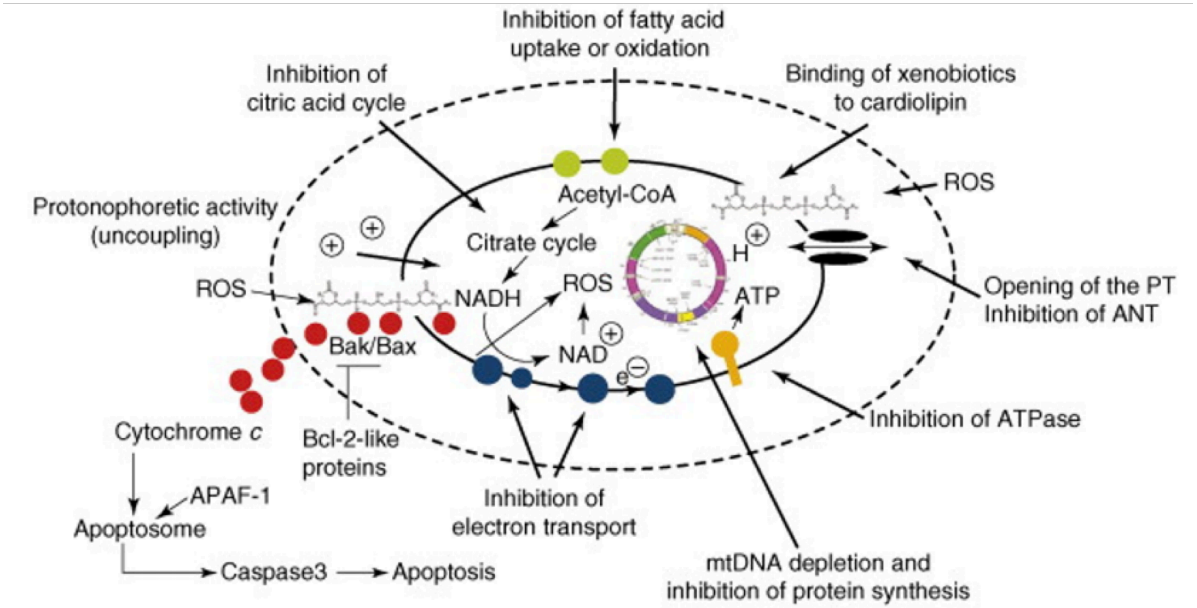
Eyes

vision loss, ptosis, optic atrophy, strabismus, ophthalmoplegia, retinitis pigmentosa

Systemic

failure to gain weight, fatigue, short stature, unexplained vomiting, respiratory problems

Prevalence of Mitochondrial Toxicity



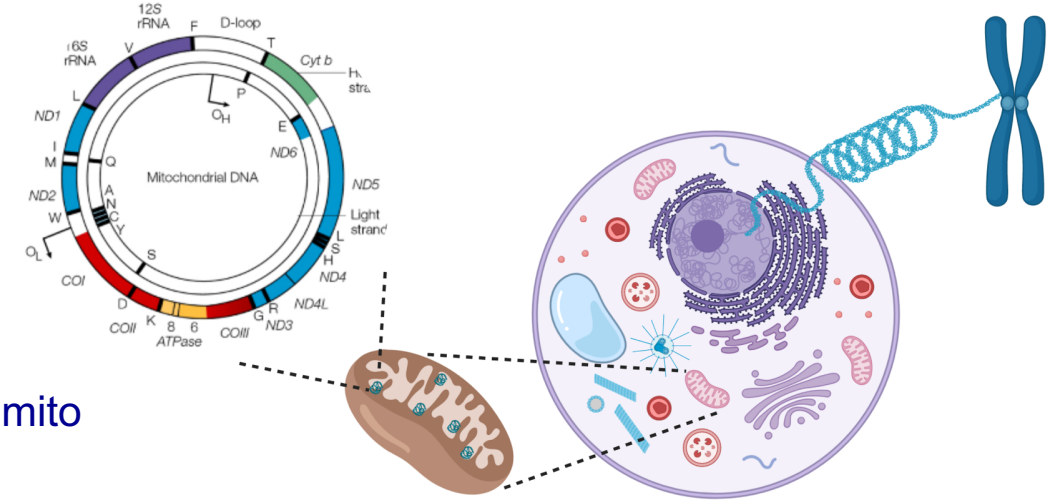
- 35% of pharmaceutically relevant molecules tested were mitotoxic; also supported by HTS studies
- Many chemical pollutants affect mitochondria, although in many cases mitochondria are not the only subcellular target
- Effects may persist long after exposure ceases
- Impact on the nuclear genes important for mito functions

Dykens & Will, 2007. Drug Discov. Today; Meyer & Chan, 2017. Toxicology

Known Mitochondrial Disruptors

- Rotenone (piscicide or fish poison) — complex I inhibitor, ROS
- MPTP — bioactivation to MPP^+, which accumulates in mitochondria and inhibits ETC, ROS
- Heavy metals (Cu, Cd, Pb, Mn, Hg, As, and Al) — ROS
- Cyanide — complex IV inhibitor
- Azidothymidine (an anti-HIV drug) — accumulates within the mitochondrial intermembrane space where it disrupts the ATP/ADP translocator and enhances the production of ROS
- Emerging: polycyclic aromatic hydrocarbons, particulate air pollutants, heavy metals, endocrine-disrupting chemicals, pesticides, nanomaterials

Mitochondrial DNA (mtDNA)



99% genes function in mito are encoded in nDNA

	nuclear DNA	mtDNA
size	3.3 x 10^9 bp	16,569 bp
number of genes	20,000 – 30,000	37 (13 polypeptides, 22 tRNAs, 2 rRNAs)
chromosomes	22 autosomes, 2 sex	1 (polyploidy, 2-10 per organelle)
coding DNA	~ 3%	93% (no introns, a non-coding region)
inheritance	autosomal	maternal
transcription	individual gene	polycistronic
DNA replication	cell cycle dependent	independent of cell cycle
half-life	30-40 days	a few days

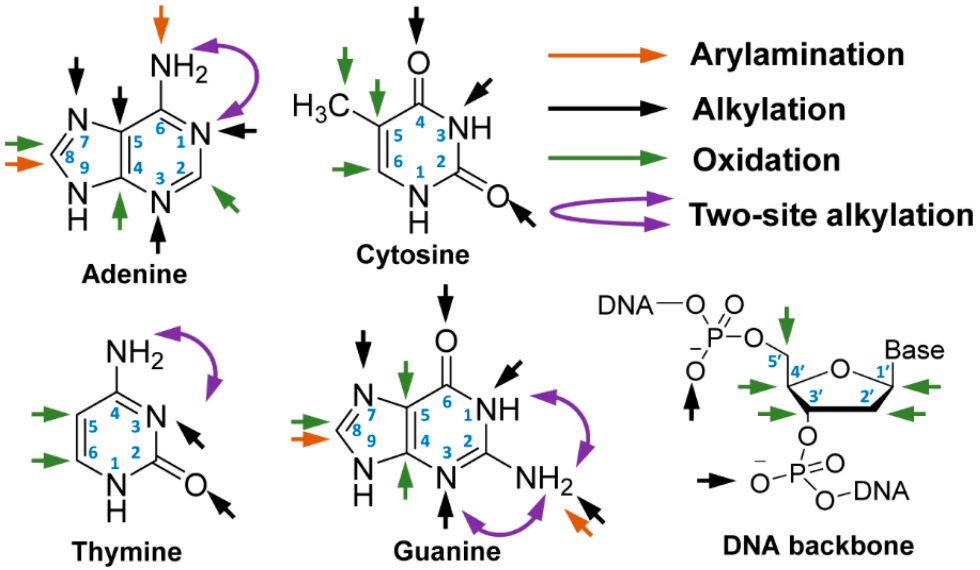
Chemicals Targeting mtDNA

Source of environmental contamination	Compound	Mitochondrial changes		References
		Organelle level	mtDNA level	
Pesticides	rotenone		block DNA polymerase ↓ replication	Sanders et al. (2014)
	organophosphorous compounds, tri-ortho tolyl phosphate, triphenyl phosphite, parathion	↑ROS changes in ΔΨm		Carlson and Erich (1999)
PCBs	PCB quinone metabolite PCB29-pQ	↑ ROS ↓ ΔΨm induction of apoptosis		Xu et al. (2015)
	Aroclor-1254	↓ ΔΨm		Cocco et al. (2015)
Dioxins	dibenzofuran	↓phosphorylative efficiency		Duarte et al. (2013)
	TCDD	↓ ΔΨm	↓mtDNA copy number	Biswas et al. (2008)
		↑ ROS	↑ 8-OHdG levels mtDNA deletion	Wan et al. (2014) and Chen et al. (2010)
Metals/metalloids	Cd	↑ ROS apoptosis		Bertin and Averbeck (2006)
	Cd, Hg, Cu	↑ ROS ↓ΔΨm		Belyaeva et al. (2012)
	As_2O_3		↓mtDNA copy number mtDNA deletion	Zhang et al. (2011)
	Methyl Hg	apoptosis	Point mutations in the D-loop	Wang et al. (2016)
Air pollutants	Diesel exhaust Ultrafine particles	↓ΔΨm Apoptosis ↓mitochondrial mass		Xia et al. (2004)
	B[a]P	↑ ROS		Kowaltowski et al. (2009)
		Changes in the mt-proteome	↑mtDNA copy number	Kim et al. (2014)
			DNA lesions but no point mutations/deletions	Valente et al. (2016)
	NO_x	↓ATP production ↑ ROS		Yan et al. (2015)
	SO_2	↑ATP production ↑ ΔΨm	↑mtDNA copy number	Qin et al. (2012)
	SO_2 + B[a]P	↓ΔΨm Apoptotic/anti-apoptotic signals at different post-exposure times		Qin et al. (2015)
Algal toxins	Microcystin-LR	↑ ROS apoptosis	Changes in mtDNA copy number	Chen et al. (2013)
			Alter mtDNA expression	Li et al. (2016a, b)

Zolkipli-Cunningham & Falk 2017. Toxicology

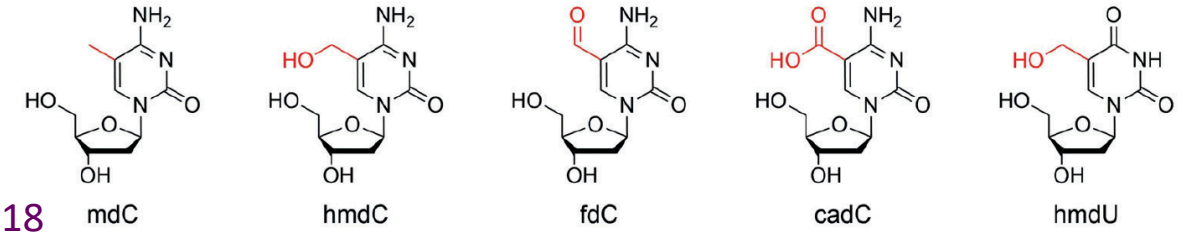
Chemistry of DNA Modifications

A broad spectrum of DNA lesions derived from environmental chemicals



Liu & Wang, Chem Soc Rev 2015
Guo & Turesky, High-Throughput 2019

> 50 kinds of endogenous DNA base modifications



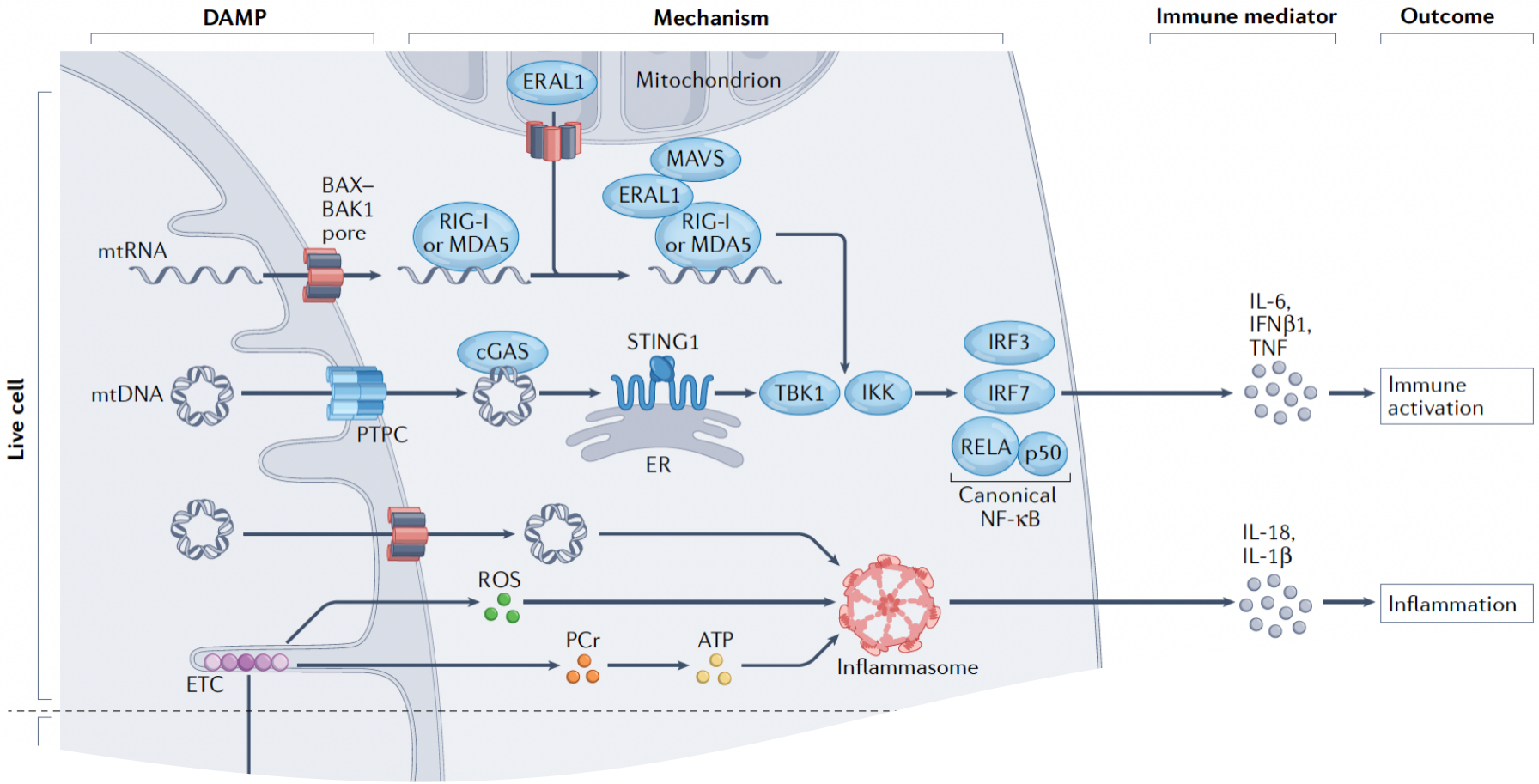
Carell, Angew Chem Int Ed 2018

Effects of Genotoxic Stress on mtDNA

- mtDNA lesions form at comparable or higher levels than nDNA
- Resistant to an increased mutation load with known mutagens or repair deficient conditions (OGG1 or MUTYH KO mice; benzo[a]pyrene or N-ethyl-N-nitrosourea (ENU) exposed mice; aflatoxin B1 exposed *C. elegans*)
- Change in mtDNA copy number varies with different chemical exposures
- Release of mtDNA into the cytoplasm to trigger immunological and inflammation pathways
- An emerging role of mtDNA as a genotoxic stress sensor

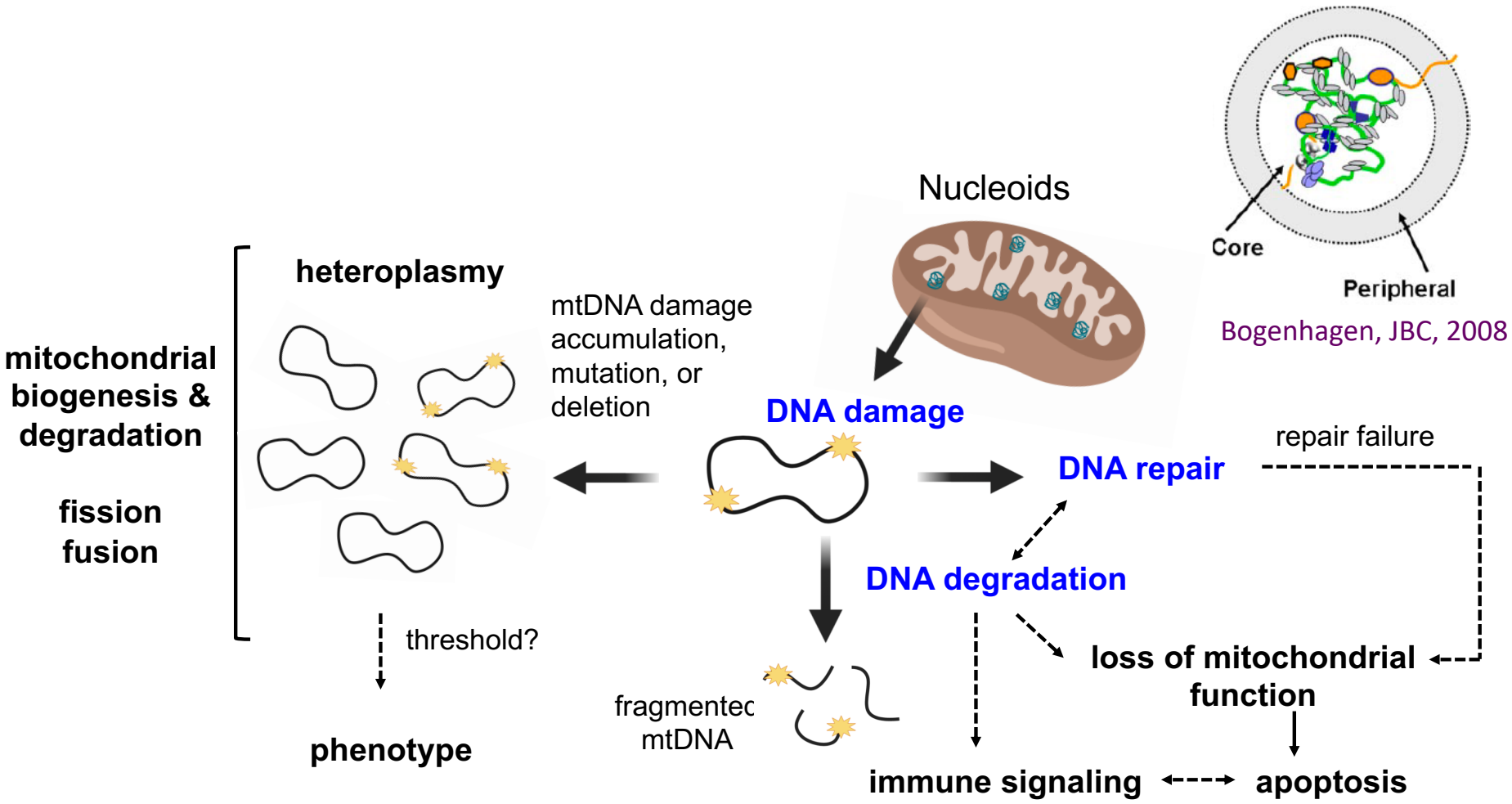
Main Mechanisms of Mito DAMP Signaling

Damage-associated molecular pattern (DAMP) molecules: nucleic acids, small metabolites, peptides



Marchi et al. Nat Rev Immunol. 2023

mtDNA Damage, Repair and Turnover



mtDNA cellular stress codes?

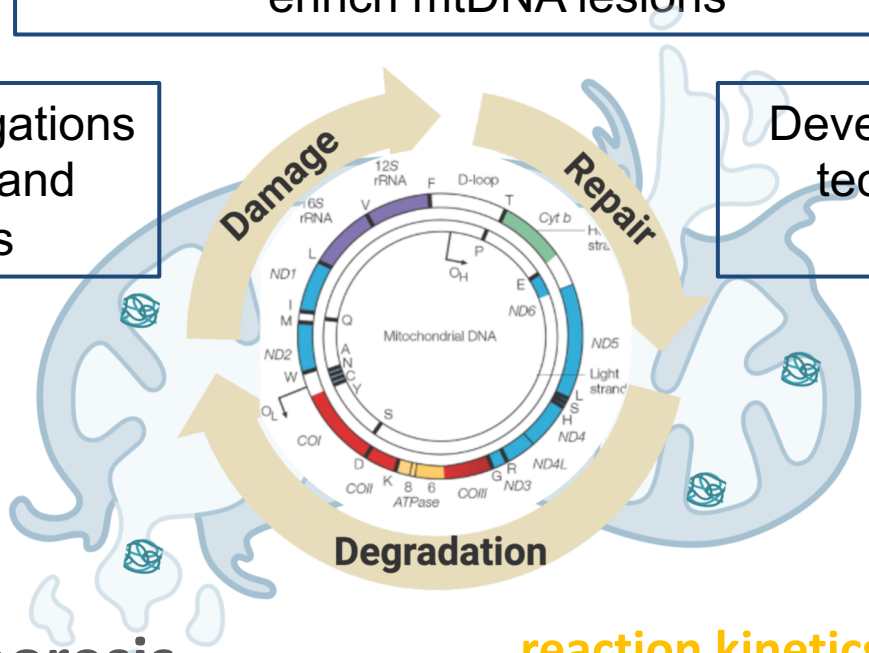
Zhao & Sumberaz, Chem Res Toxicol, 2020
Zhao, The Enzymes, 2019

Chemical Biology and Enzymology of mtDNA

Design chemical probes to manipulate and enrich mtDNA lesions

Mechanistic investigations using enzymatic and cellular assays

Develop DNA sequencing technologies for DNA modifications



gel electrophoresis

reaction kinetics

immunoassays

DNA binding assays

quantitative and mechanistic

recombinant protein

chromatography

enzymology

mass spectrometry

structural biology

purification

computer simulations

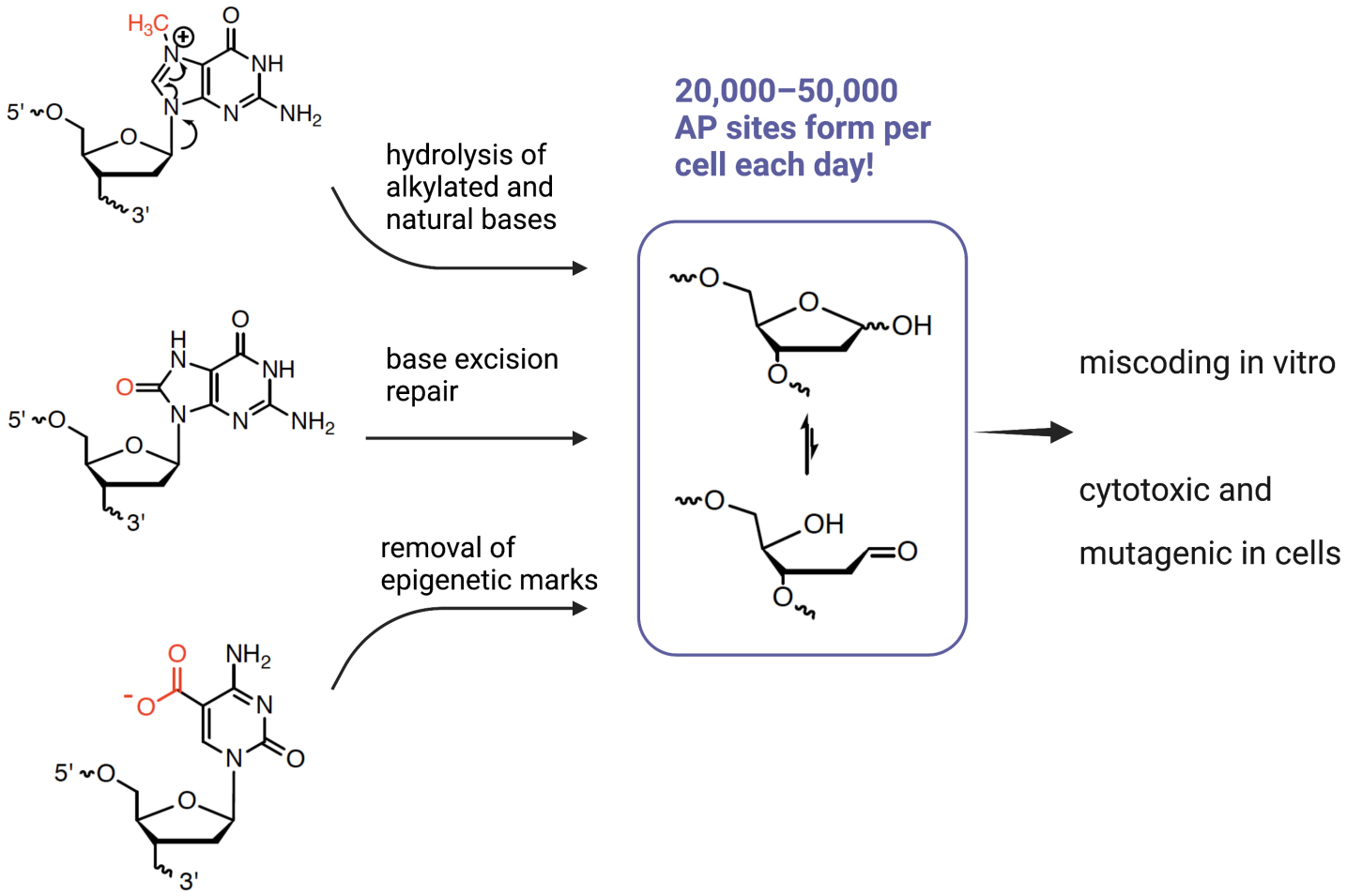
molecular biology

cell biology

separation

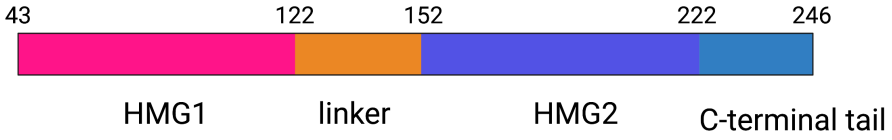
bioinformatics

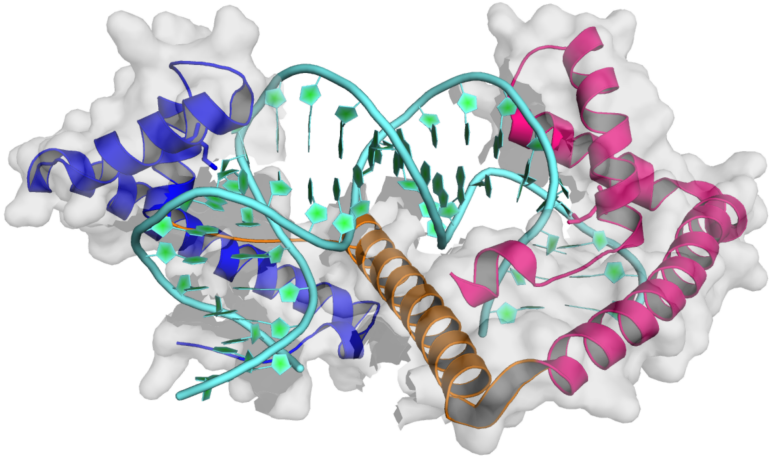
Abasic (AP) Sites: Abundance and Biological Importance



Lindahl, Biochem 1972; Swenberg, Toxicol. Sci. 2011; Kohli & Zhang, Nature 2013; Krokan, Cold Spring Harb. Perspect. Biol. 2013

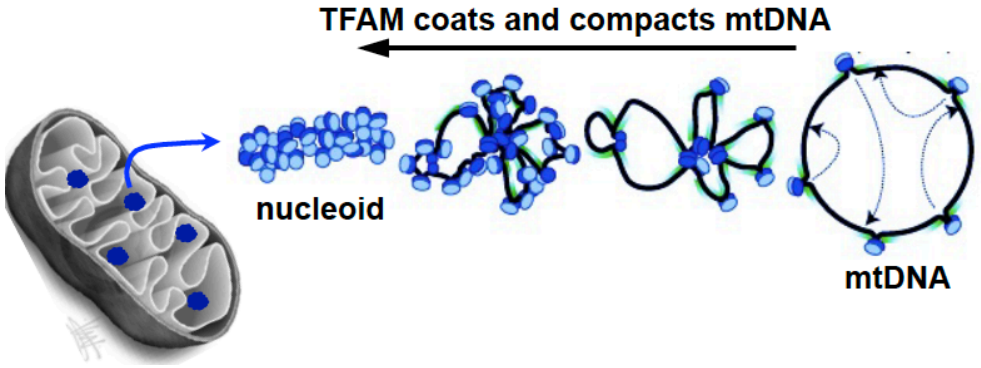
Mitochondrial Transcription Factor A (TFAM)





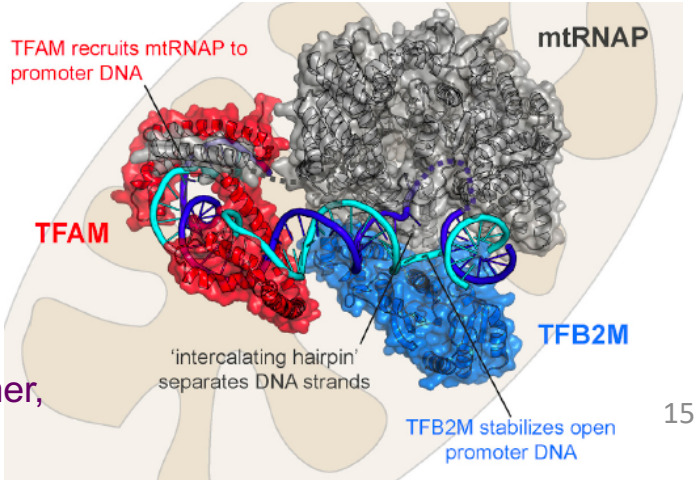
Sola, NSMB, 2011
Chan, NSMB, 2011
Chan, Nat Commun, 2014

DNA Packaging



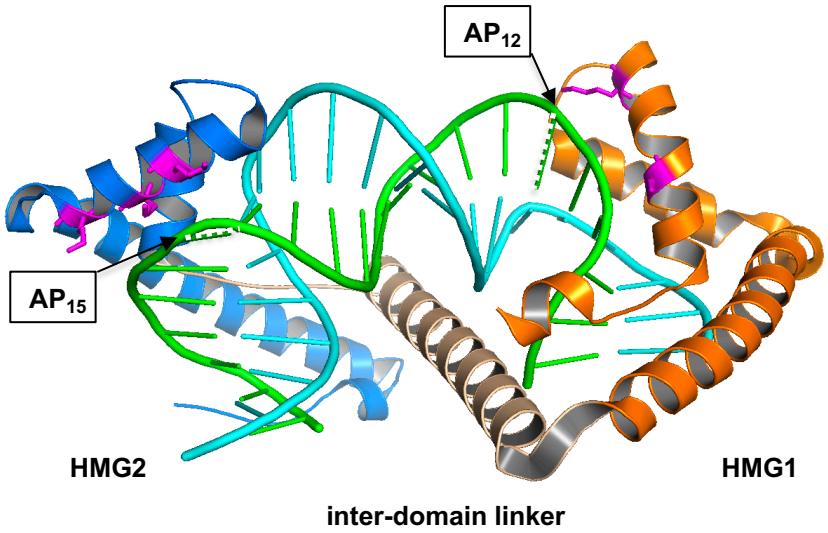
Clayton, MCB, 1988
Clayton, Science, 1991

Transcription Activation



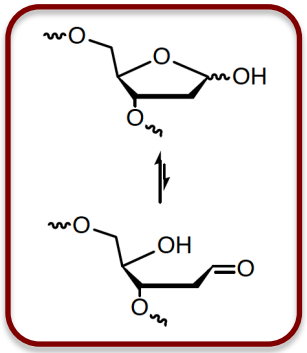
Temiakov & Cramer, Cell, 2017

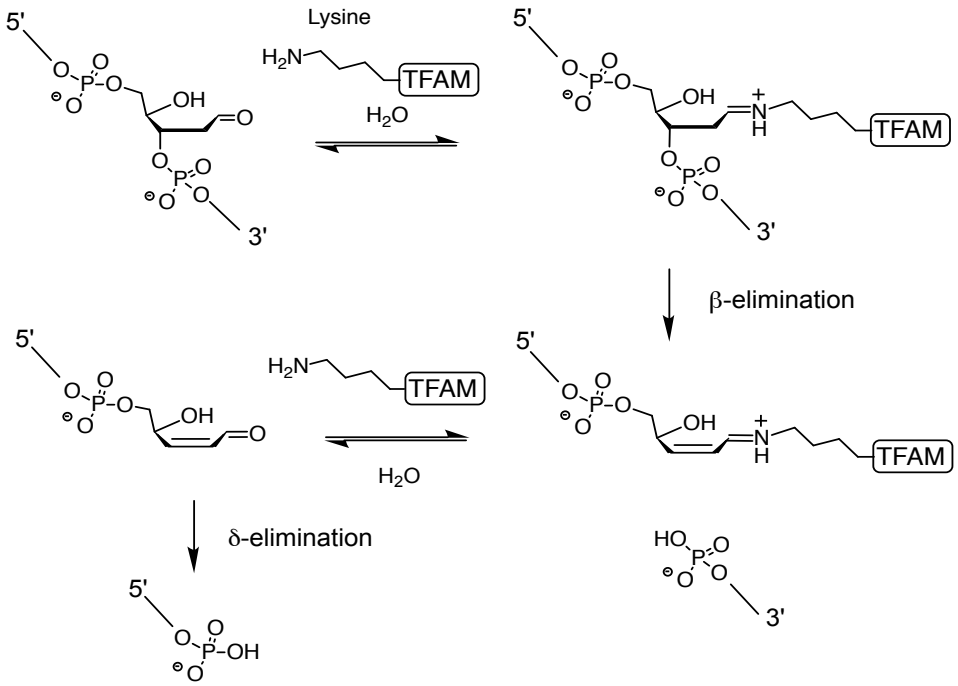
TFAM Promotes AP-DNA Cleavage

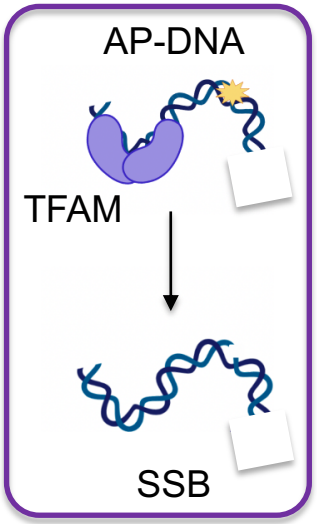


0.2-3 AP/10^5 bp in mtDNA

hundreds/cell





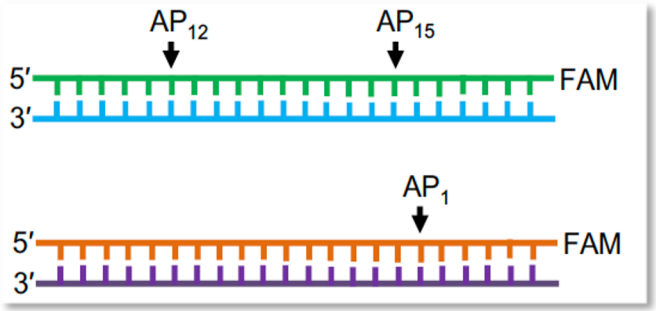


Dr. Wenyan Xu
Now - Eurofins

Xu et al. *PNAS*, 2019; Xu et al. *Nucleic Acids Res*, 2023

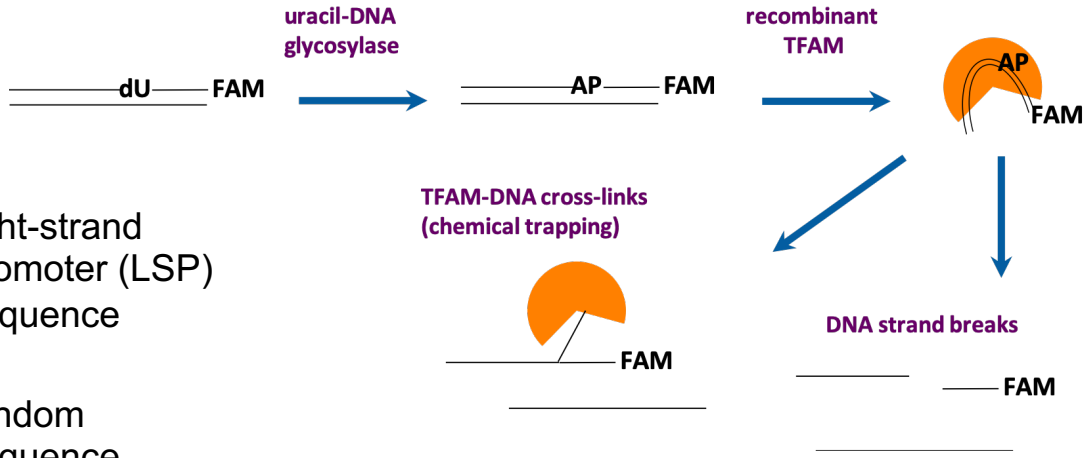
Experimental Design

AP-containing DNA substrates

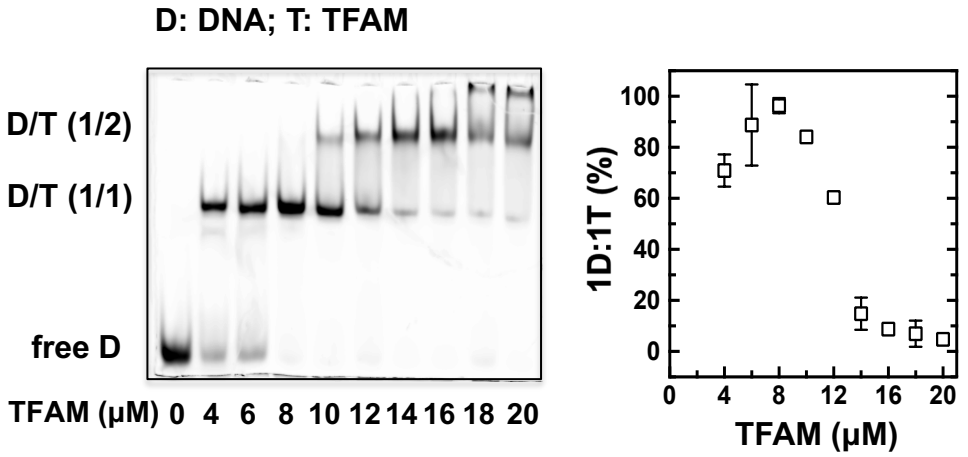


light-strand promoter (LSP) sequence

random sequence



TFAM:DNA binding stoichiometry



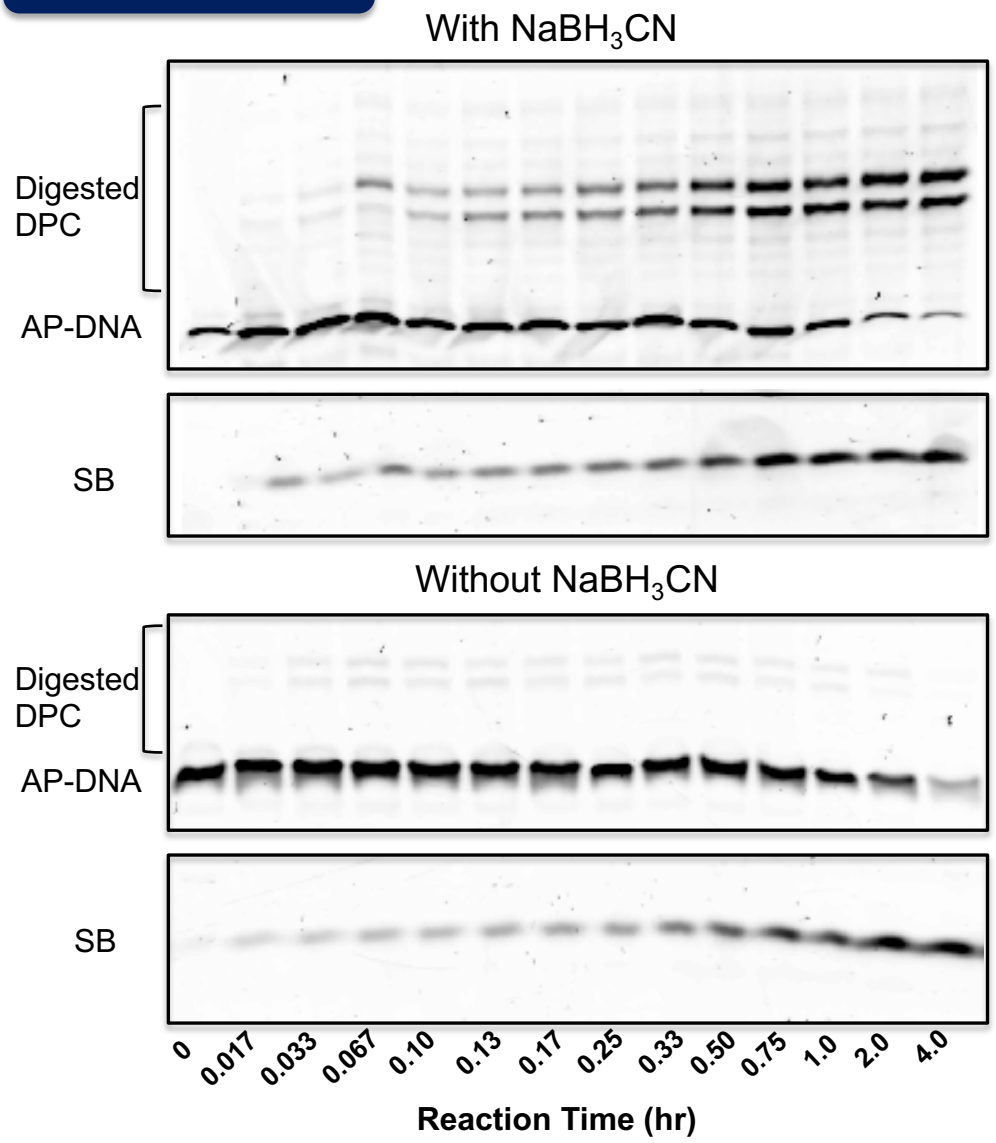
Riley Boyd

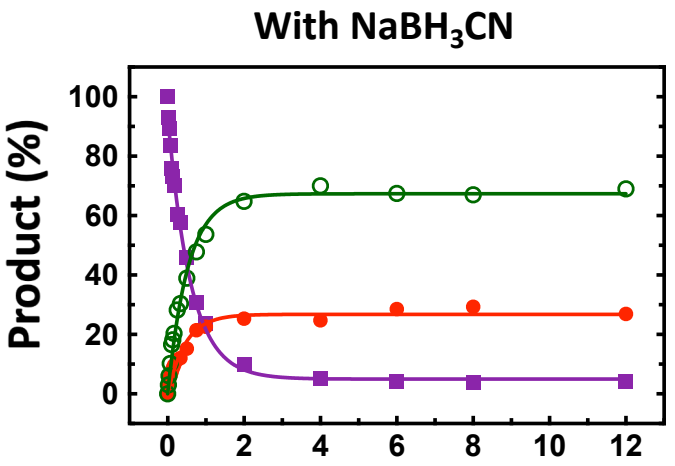
Dr. Wenyan Xu

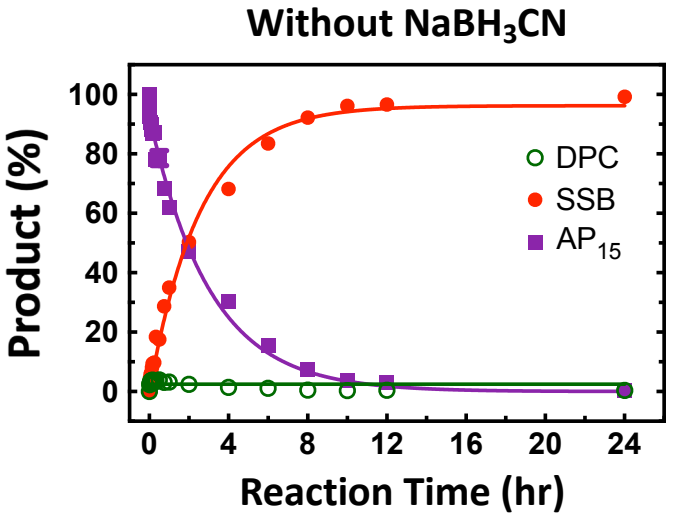
Xu et al. PNAS, 2019

Quantification of DPCs and SSB

Denaturing PAGE





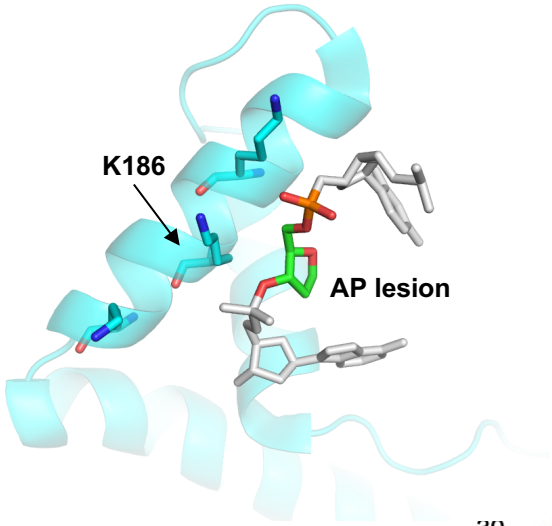


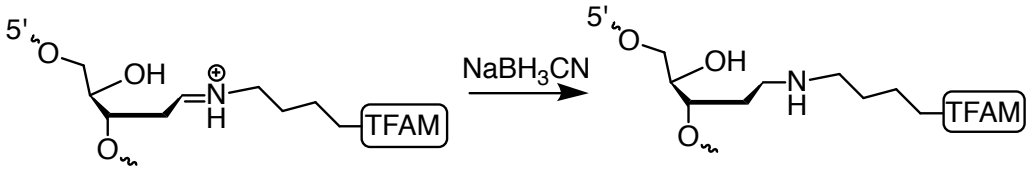
TFAM Reduces the Stability of AP-DNA

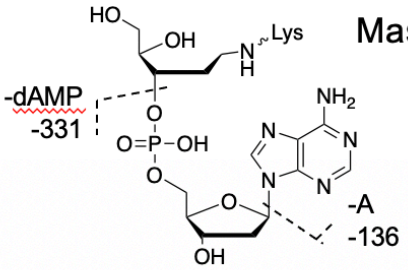
AP Stability as a Function of Position

		TFAM-DNA complex		free DNA	
AP position	$NaBH_3CN$	k_{dis} (10^{-5} s^{-1})	$t_{1/2}$ (h)	$t_{1/2}$ (h)	
AP_{12}	−	9.1 ± 0.7	2.1 ± 0.2	480 ± 50	~230-fold reduction
AP_{12}	+	12 ± 1	1.6 ± 0.2	−	
AP_{15}	−	9.1 ± 2.1	2.3 ± 0.4	2800 ± 100	~ 1200-fold reduction
AP_{15}	+	46 ± 8	0.4 ± 0.1	−	
AP_1	−	3.4 ± 0.4	5.7 ± 0.7	980 ± 120	~170-fold reduction
AP_1	+	7.2 ± 0.2	2.7 ± 0.1	−	
AP_{15} (N_α-acetyl-lysine)	+	0.068 ± 0.010	607		

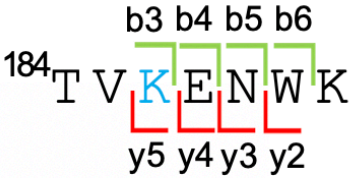
Lys Residues Facilitate AP-DNA Cleavage







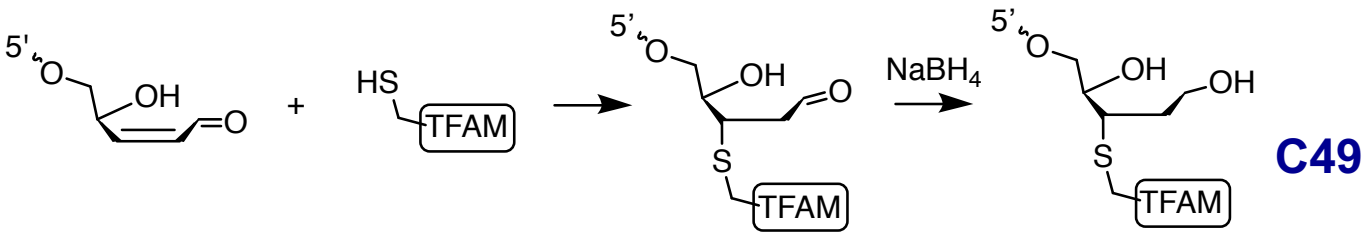
Mass adduct 431



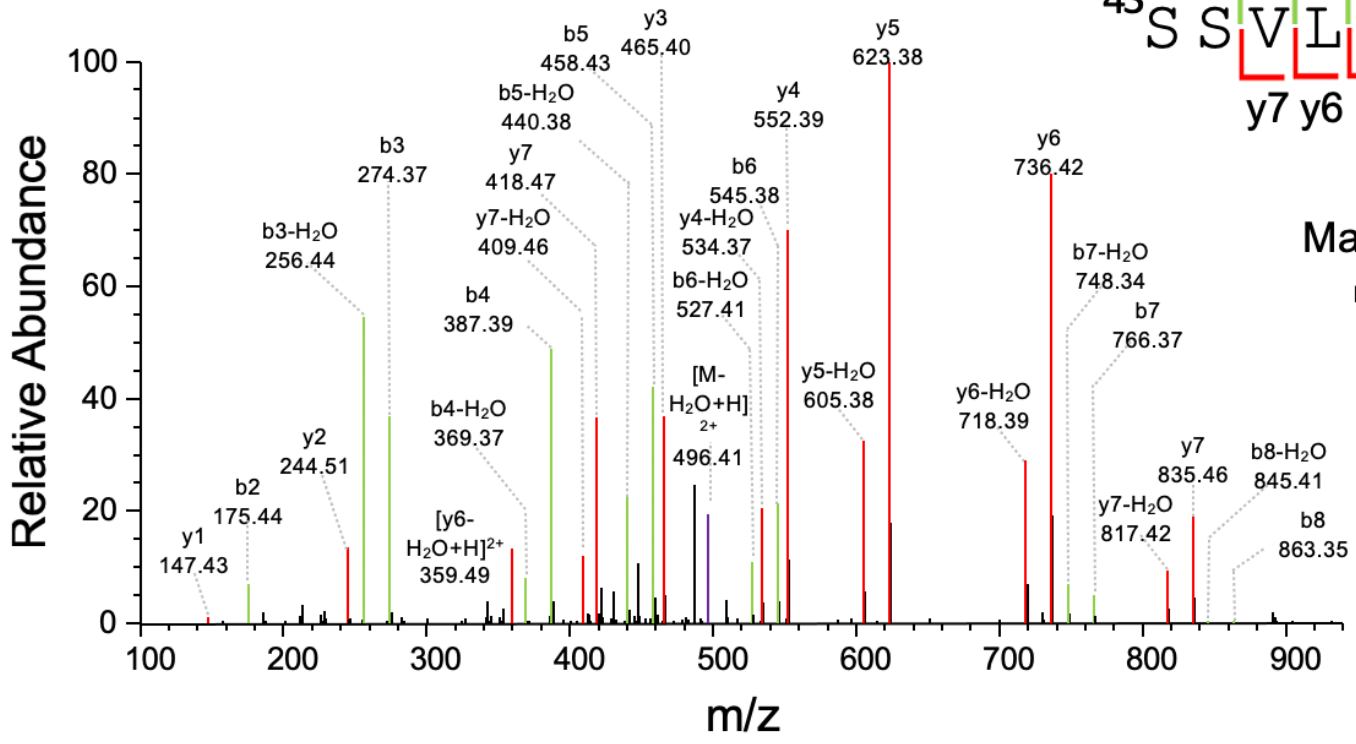
Jin Tang
Now – specialist UCSF

Tang et al. *Anal Chem*, 2021

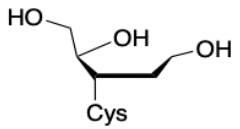
Cys Residues Form Stable TFAM-DPCs



C49

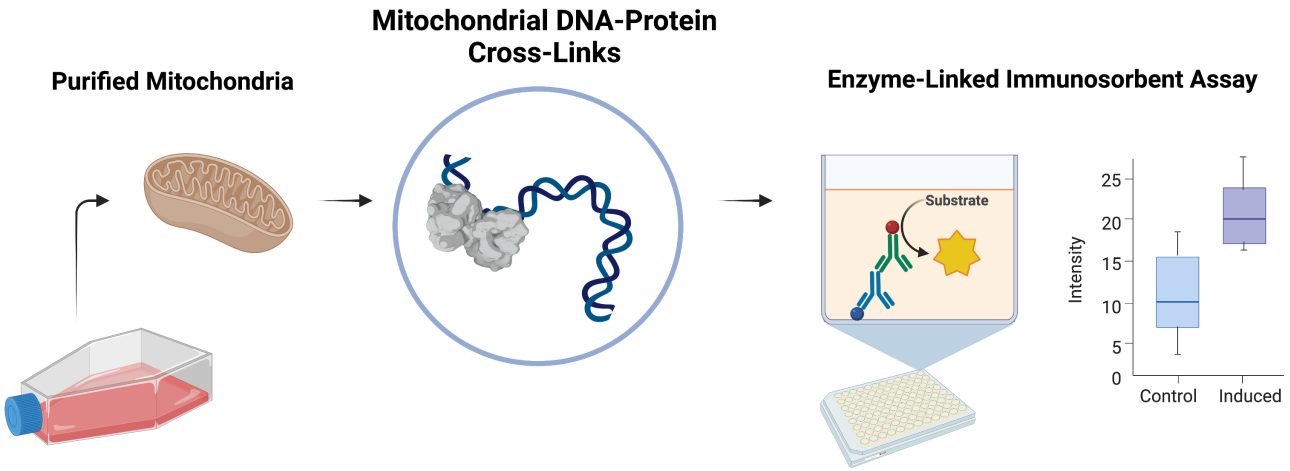


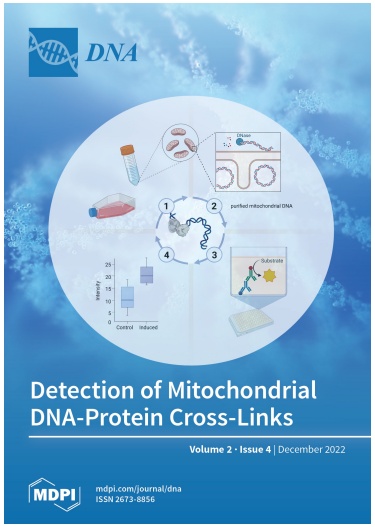
Mass adduct 118



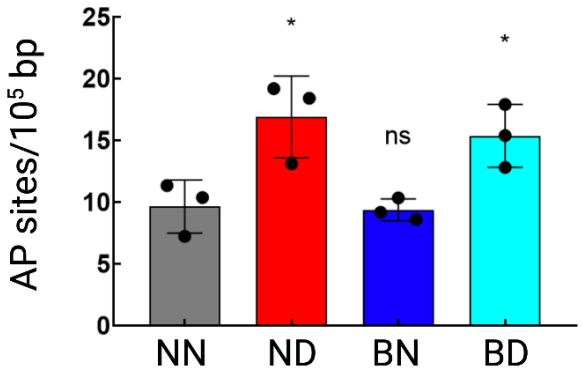
Xu et al. *Nucleic Acids Res*, 2023

TFAM-DPCs Form in Cells and Regulated by GSH

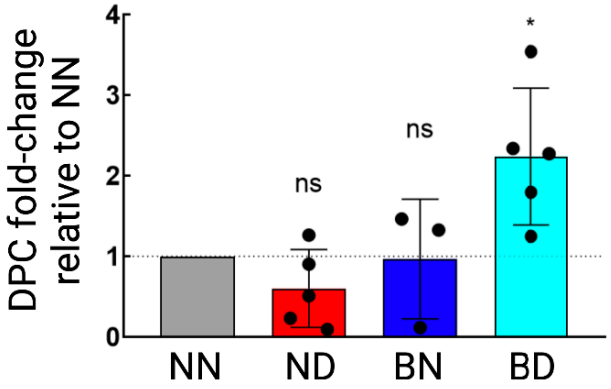




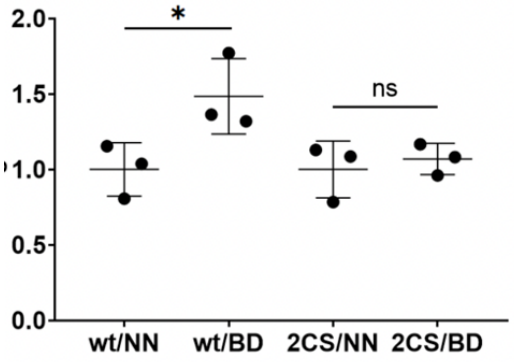
Quantification of AP sites



TFAM-DNA cross-links

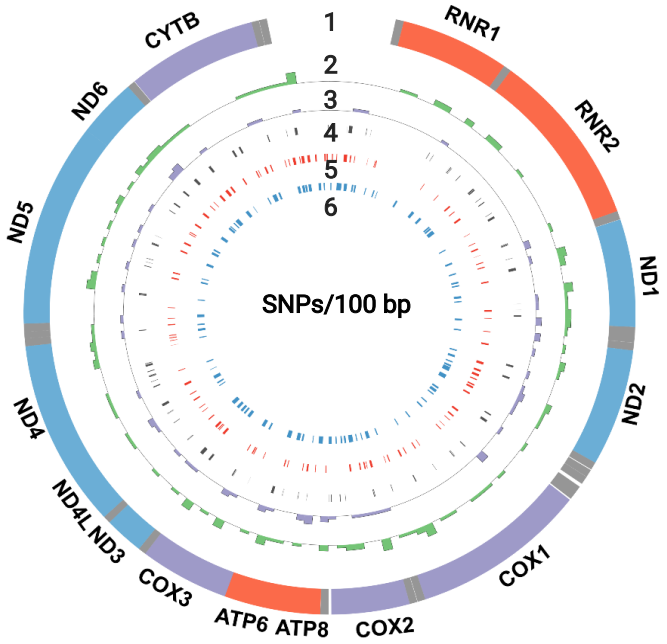


Role of Cys Residues



Xu et al. *Nucleic Acids Res*, 2023
Xu et al. *DNA*, 2022

AP Positions Correlate with “low TFAM” Sites



1 - mtDNA map
2 - Dox & APE1 inhibitor
3 - untreated control
4 - mtDGF
5 - high TFAM sites
6 - low TFAM sites

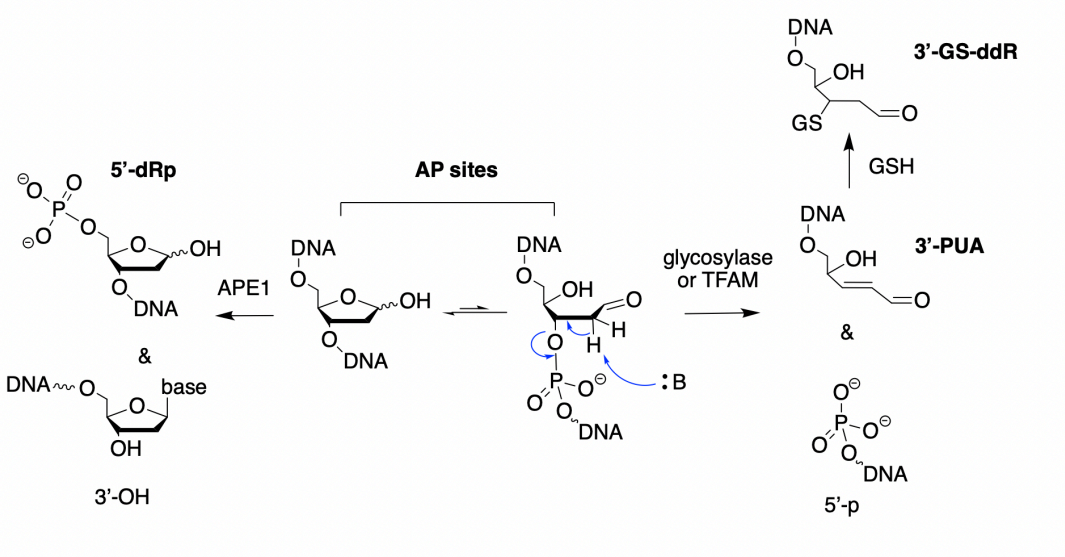
Dr. Chaoxing Liu
Now – PI, Sun Yat-Sen Univ.

- Correlations with low TFAM sites corroborate the role of TFAM in promoting AP-DNA cleavage.
- Correlations with G4

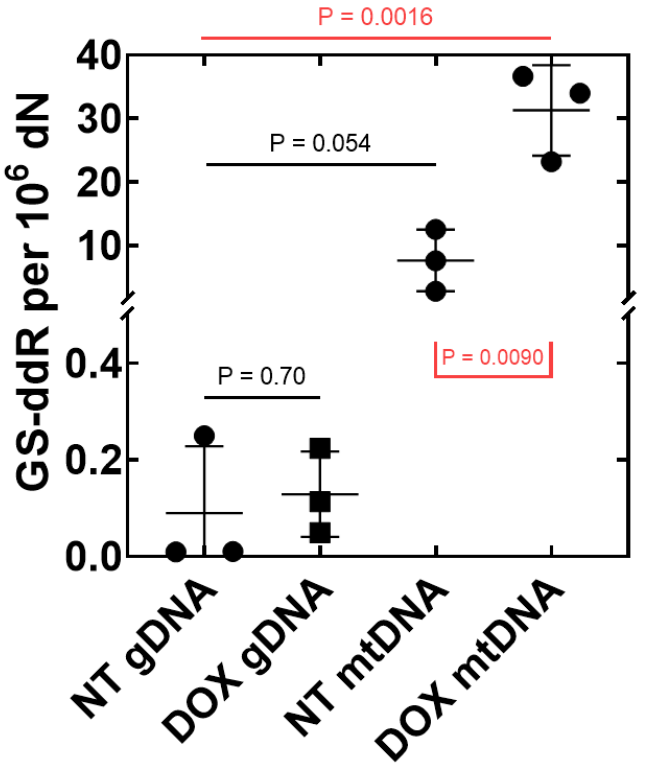
	Control	Induced
No. of AP locations	65	125
correlation with “low TFAM” sites	22 (34%), p = 0.038	42 (34%), p = 0.001
correlation with “high TFAM” sites	8 (12%) , p = 0.71	6 (4.8%) , p = 0.0003

Liu et al. *Nucleic Acids Res*. 2023

Abundant GSH-DNA Adducts in Mitochondria



GSH-DNA adducts form at much higher levels in mtDNA relative to nDNA

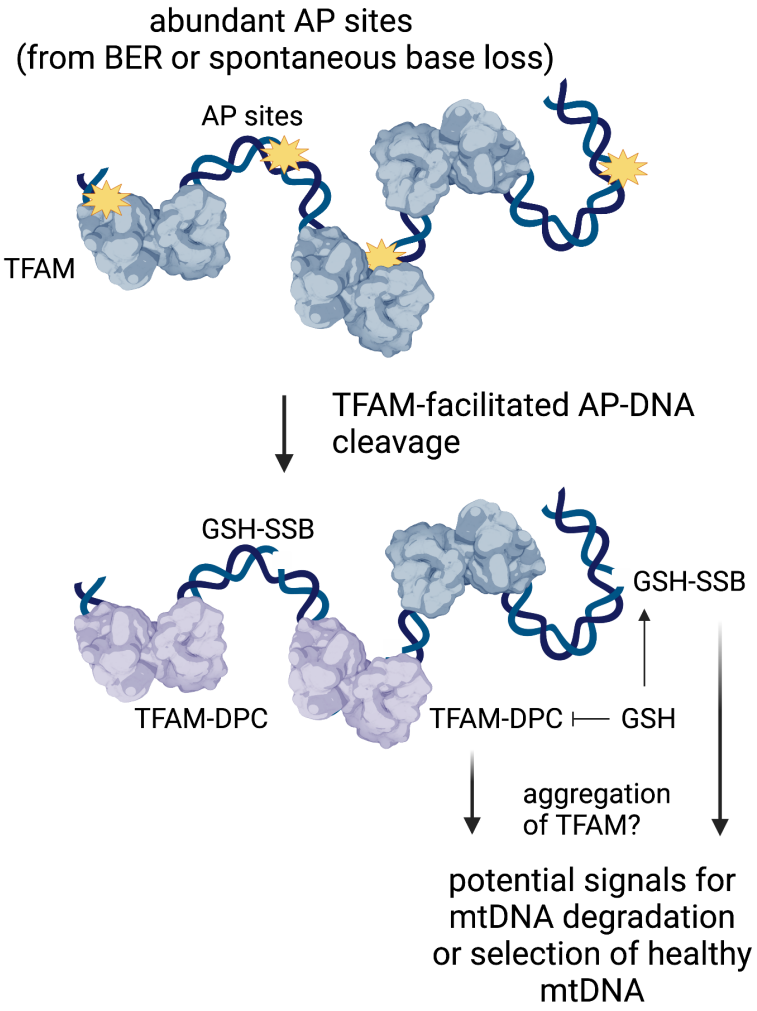


Yu Hsuan Chen

Martin Esparza Sanchez

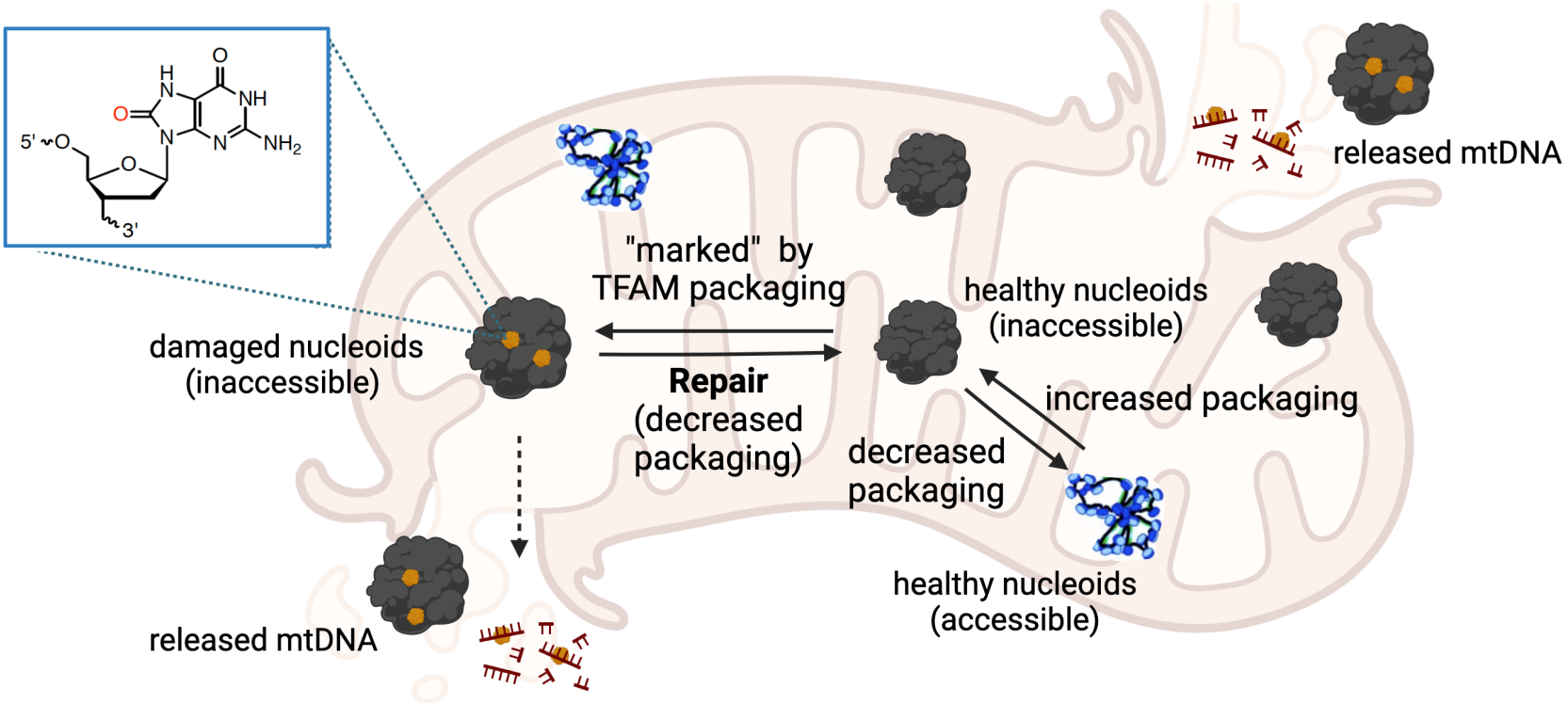
Chen et al. *in preparation*

Potential Roles of TFAM-DPCs and GSH-DNA Adducts



- Are TFAM-DPCs and GSH-DNA adducts general products from environmental chemical exposures?
- Are they triggers of mtDNA turnover?
- Are they released into the cytoplasm? If so, are they pro-inflammatory?

TFAM Regulates mtDNA Repair



- TFAM stimulates UNG1 and APE1 enzymatic turnover under optimal TFAM/DNA molar ratios
- 8-oxo-7,8-dihydro-2'-deoxyguanosine enhances TFAM-DNA binding when present in specific sequence motifs.
- Analysis of published 8-oxodG and TFAM mapping data reveals a correlation between 8-oxodG and TFAM locations in mtDNA

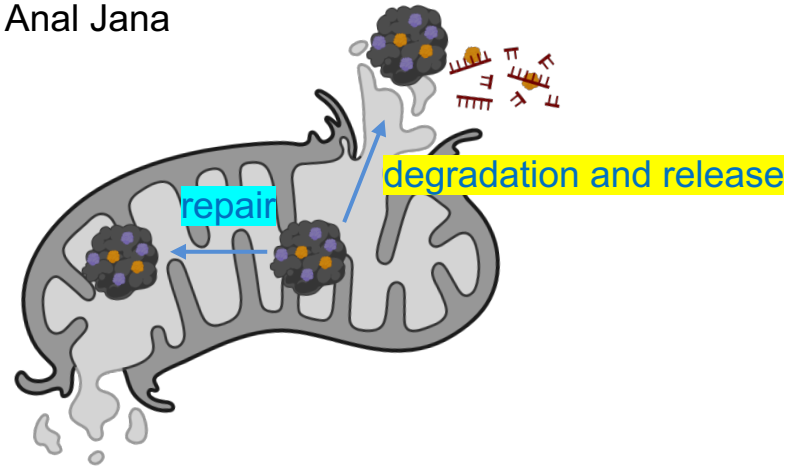
Urrutia et al. *Nucleic Acids Res*. 2024

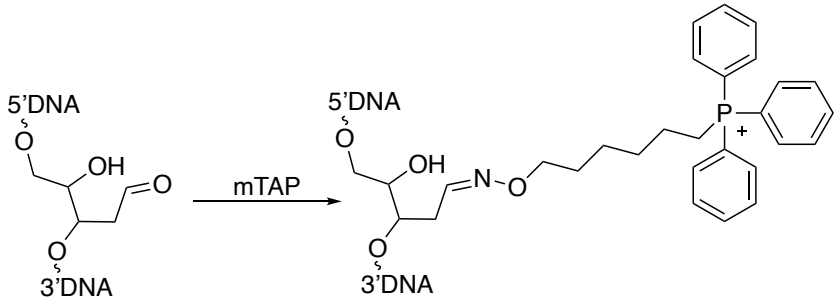
Fate of Released mtDNA

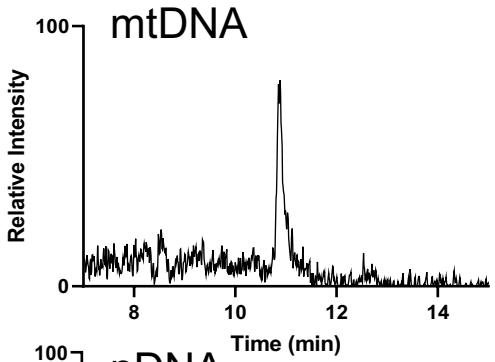
- Cytoplasm – innate immune and inflammation responses
- Circulating cell-free (ccf)-mtDNA – a systemic alarmin
- Exists in different forms – cell-free mitochondria, extracellular vesicles or free mtDNA
- Functional importance of ccf-mtDNA is controversial
- Challenges – small amounts, heterogeneity, lack of purification methods
- Recommendations: Trumpff et al. Mitochondrion 59 (2021) 225–245

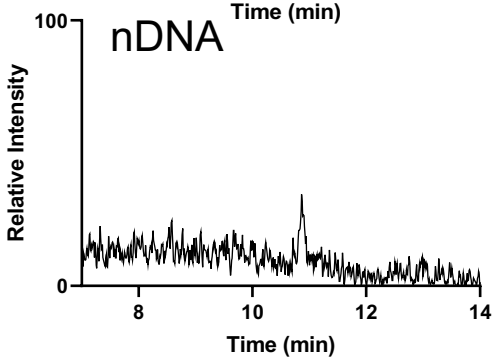
Manipulation of mtDNA Turnover

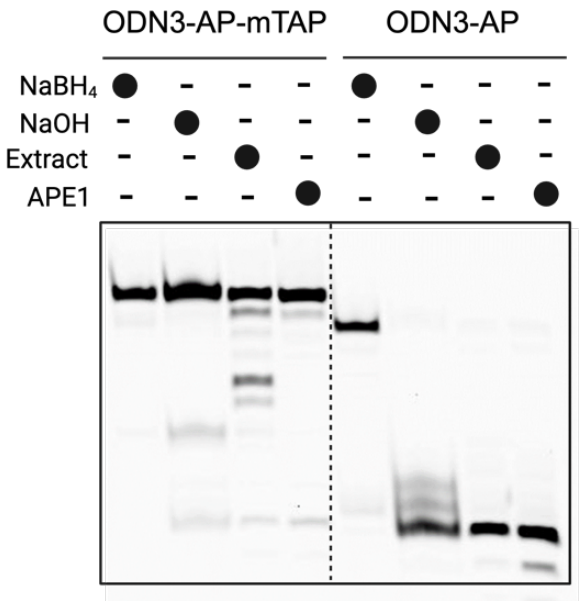
Dr. Anal Jana

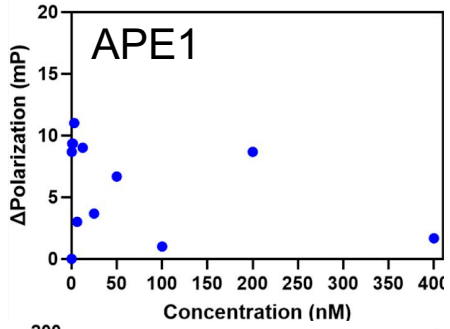


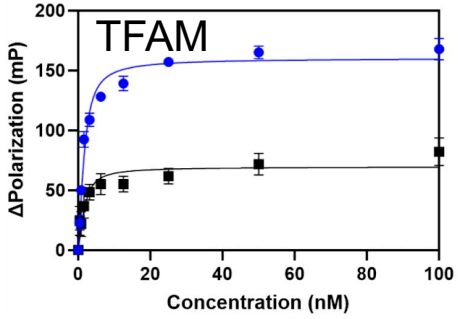






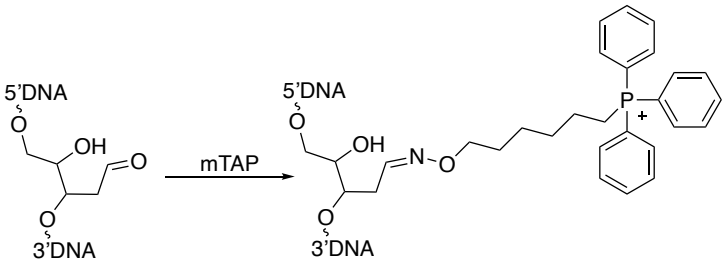


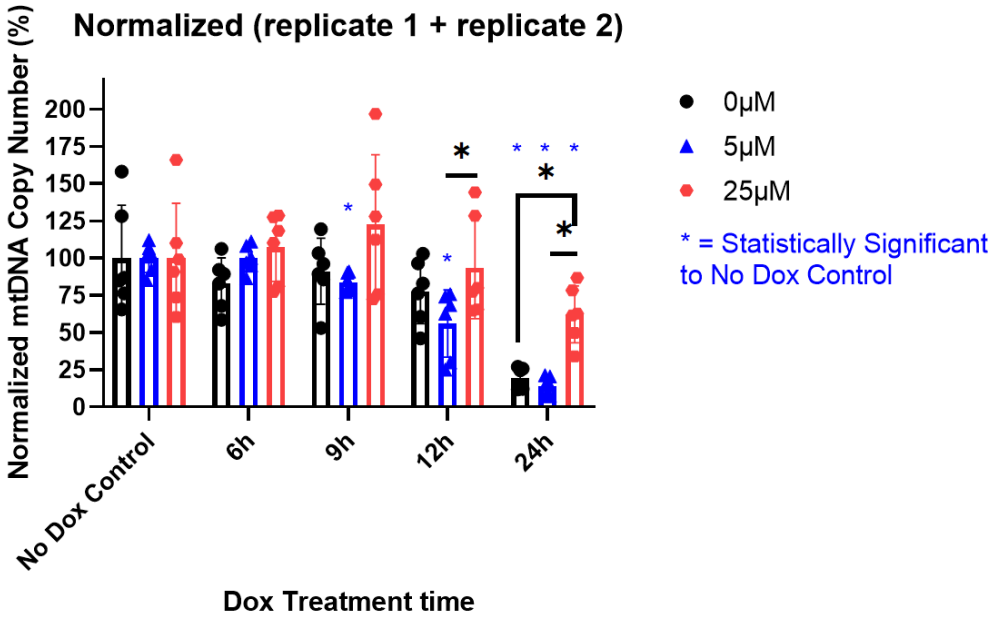


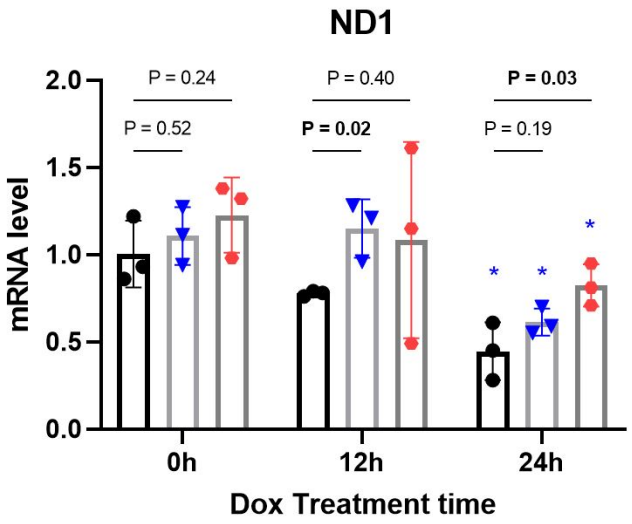


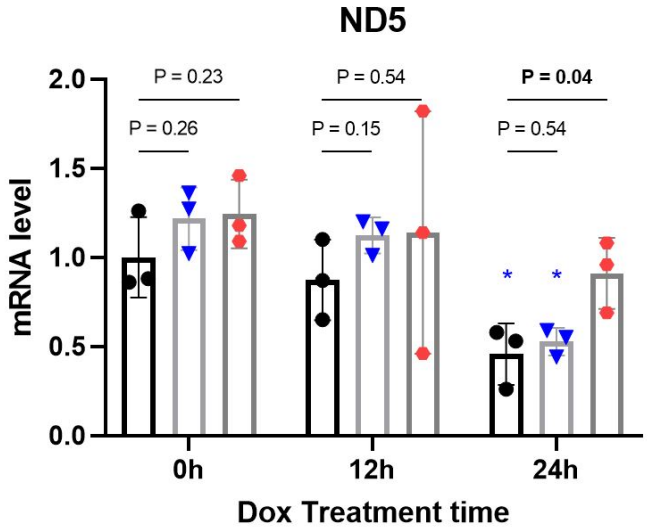
Jana et al. *in preparation*

Manipulation of mtDNA Turnover









Summary

- mtDNA has emerged as a genotoxic stress sensor
- Damaged mtDNA may contain cell stress codes
- Our studies have shown TFAM plays multiple roles in facilitating damaged mtDNA (AP-containing) and regulating the mtDNA repair
- **Mechanistic insights enable us to investigate the forms and functions of mtDNA under chemical exposures and validate certain forms of mtDNA as biomarkers**

Acknowledgement

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R21 HG012412

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