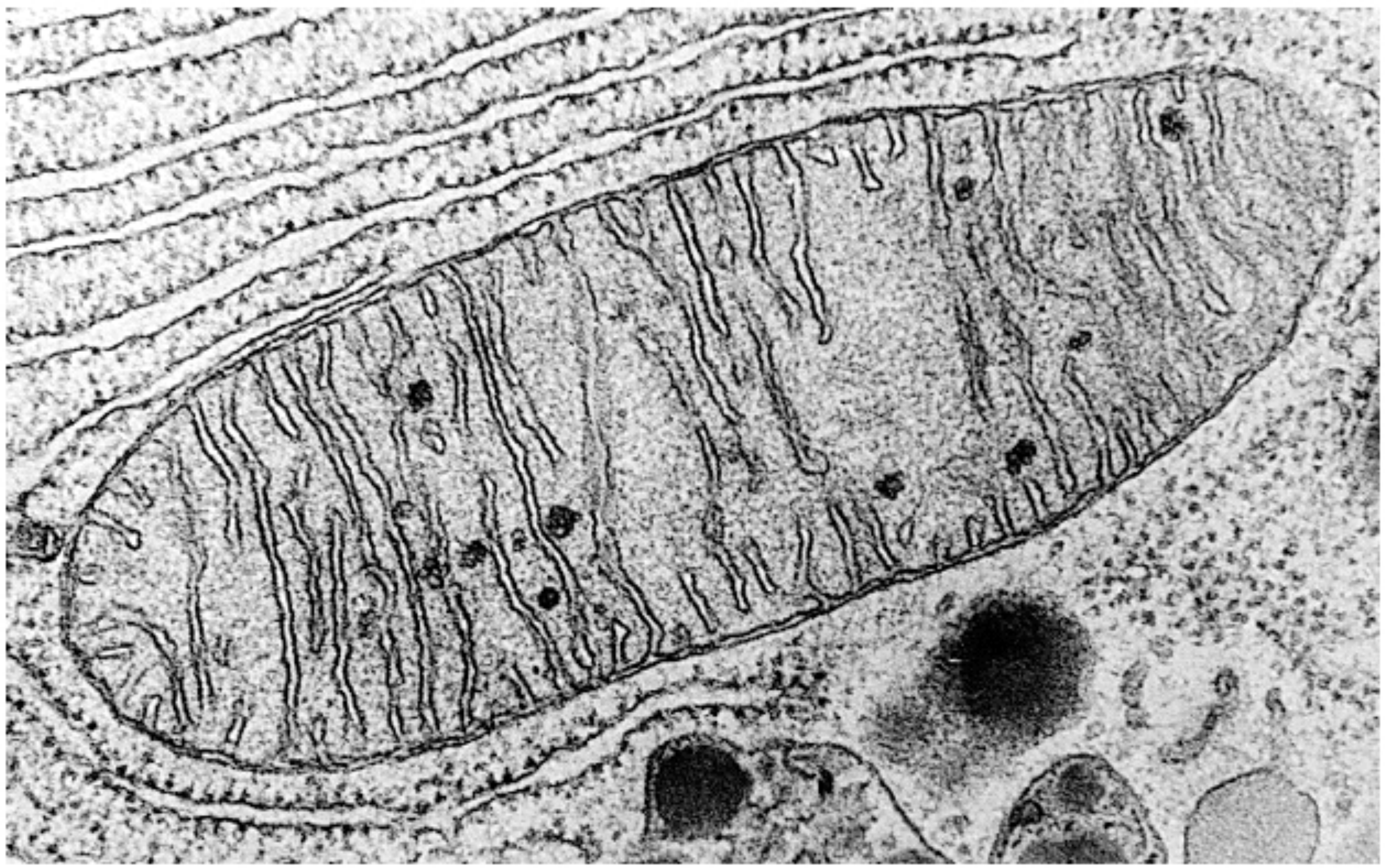
Maintaining a healthy mitochondrial genome

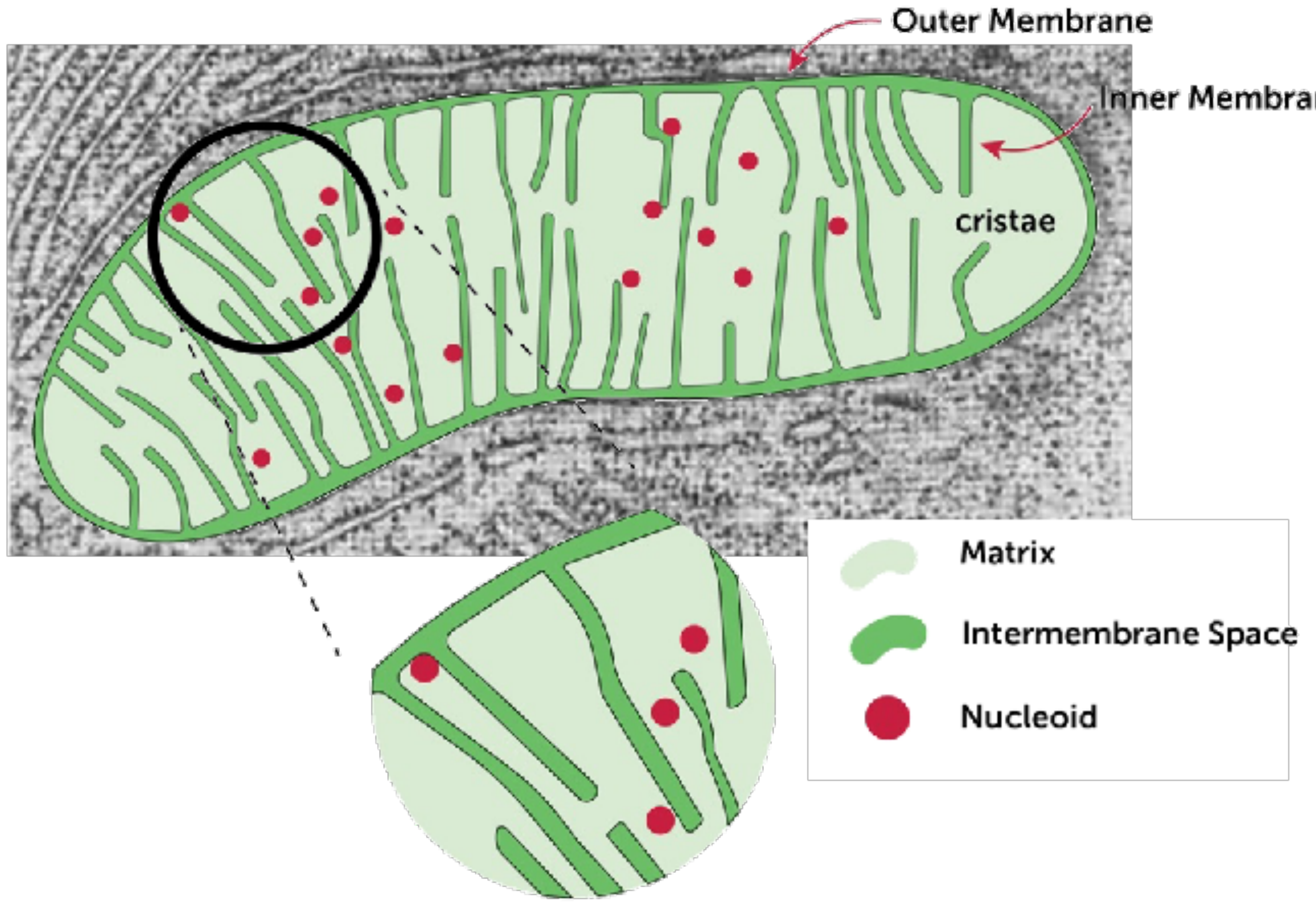


Agnel Sfeir
October 2025

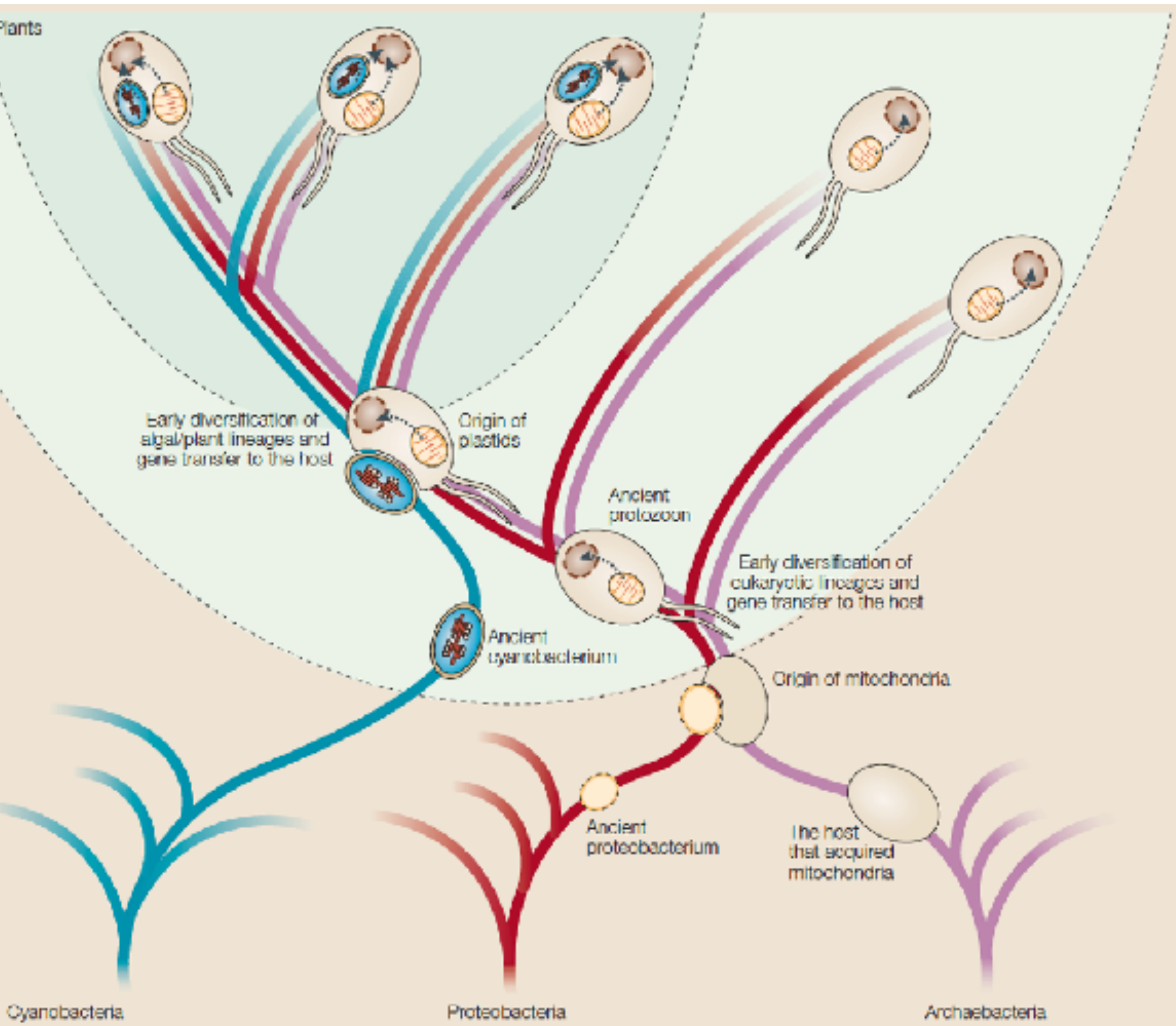
Learning Objective

- Mitochondria are semi-autonomous organelles with their own genome.
- mtDNA encodes unique genes not found in the nuclear genome.
- Mitochondria maintain independent systems for DNA replication, transcription, and translation.
- Mitochondrial function is essential for life; mutations in nuclear or mitochondrial genes cause disease.
- Clinical manifestations of mitochondrial disorders vary with heteroplasmy, mtDNA copy number, and genotype.
- mtDNA variant accumulation and selection are shaped by genetic bottlenecks and selective pressures.

Mitochondrial structure



The Origin of Mitochondria: endosymbiotic evolutions



Timmis et al., Nature Reviews Genetics 2004

J. Theoret. Biol. (1967) **14**, 225–274

On the Origin of Mitosing Cells

LYNN SAGAN

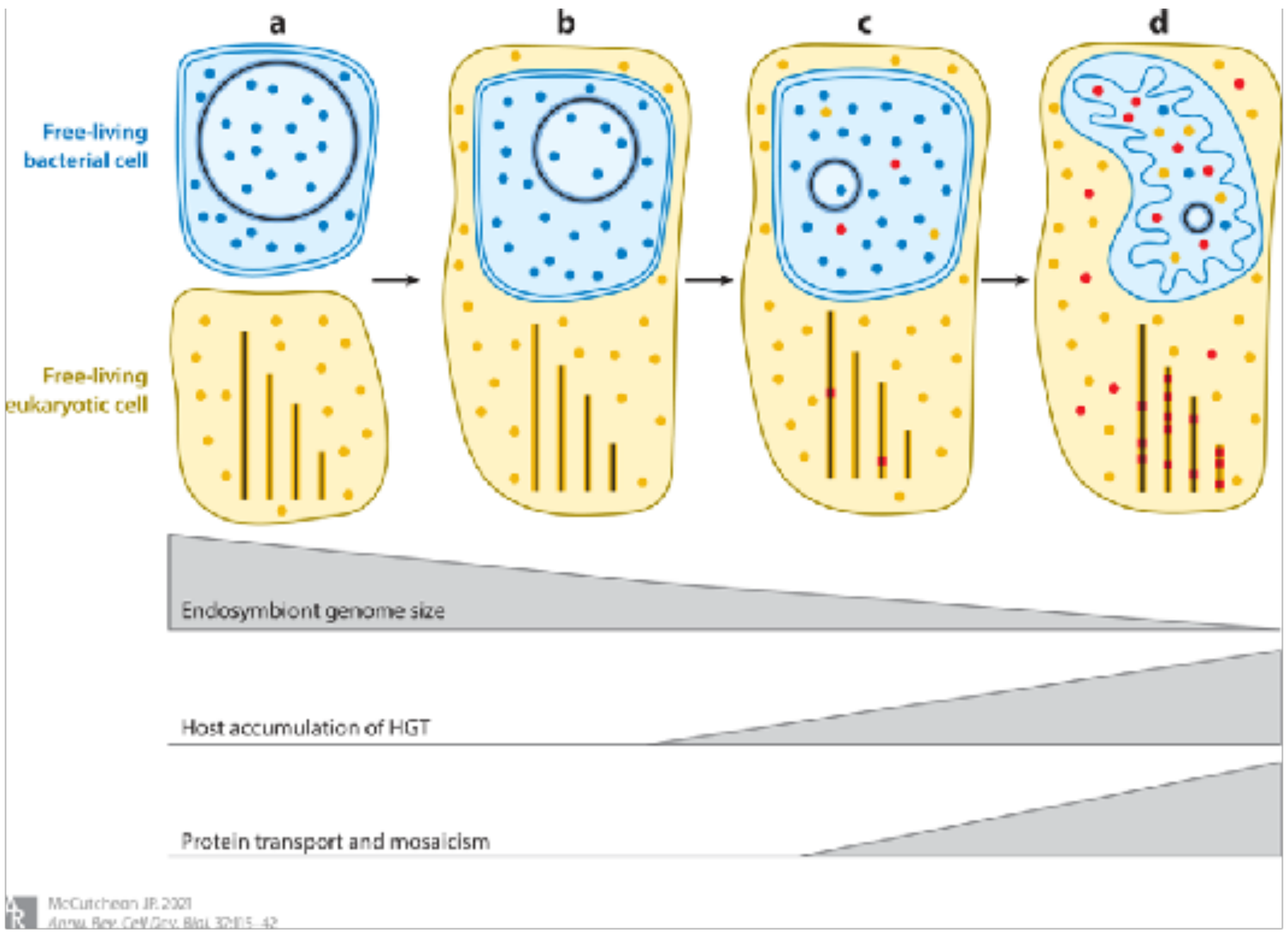
Department of Biology, Boston University
Boston, Massachusetts, U.S.A.

(*Received* 8 *June* 1966)

A theory of the origin of eukaryotic cells ("higher" cells which divide by classical mitosis) is presented. By hypothesis, three fundamental organelles: the mitochondria, the photosynthetic plastids and the (9+2) basal bodies of flagella were themselves once free-living (prokaryotic) cells. The evolu-

Cu

The Origin of Mitochondria: endosymbiotic evolutions



- **Proto-eukaryote host** (Archeabacteria)
- Endosymbiont: **aerobic α proto-bacterium**
- Nucleus Acquire endosymbiont genetic material
  - Mitochondrial Proteome: 1136+13
  - Proteins coded on mtDNA: 13
  - Percentage transferred : 98.9%
- Reduction of proto-mitochondrial genome
- Development of protein import systems to deliver proteins in the new acquired organelle.

Evidence for Endosymbiotic evolution

•Extensive phylogenetic data based on ribosomal rRNA sequencing.

•Both mitochondria and bacteria have double membranes.

•Mitochondria have their own genomes that are: Circular, With a higher ratio of G/C base pairs to A/T compared to nDNA

•Completely independent expression machineries not resembling archeabacteria.

•Lipid composition of mitochondrial membranes is closer to bacterial membranes than to eukaryotic membranes; in particular, “cardiolipin” is only present in bacterial and mitochondrial membranes.

The discovery of mtDNA

INTRAMITOCHONDRIAL FIBERS WITH DNA CHARACTERISTICS

MARGIT M. K. NASS, Ph.D., and SYLVAN NASS, Ph.D.

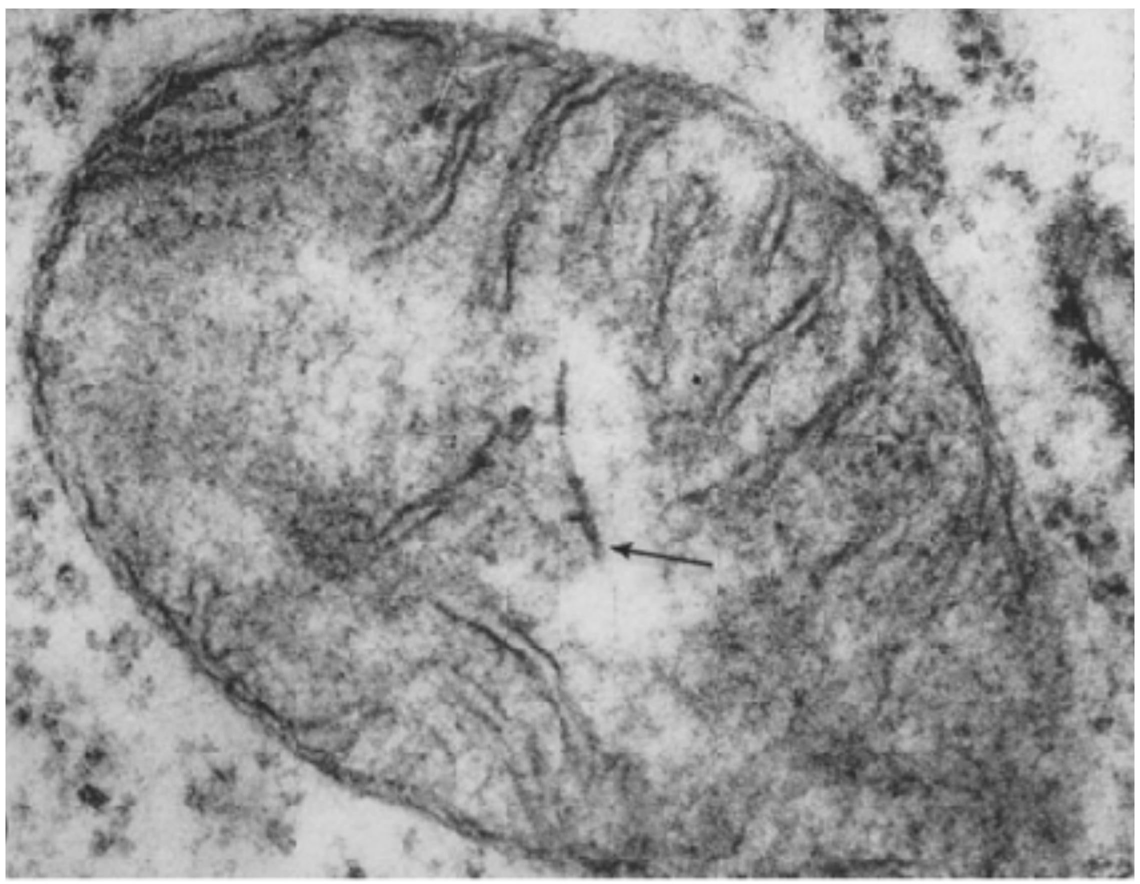
THE JOURNAL OF CELL BIOLOGY · VOLUME 19, 1963

DEOXYRIBONUCLEIC ACID ASSOCIATED WITH YEAST MITOCHONDRIA

G. SCHATZ. E. HASLBRUNNER and H. TUPPY

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

Vol. 15, No. 2, 1964



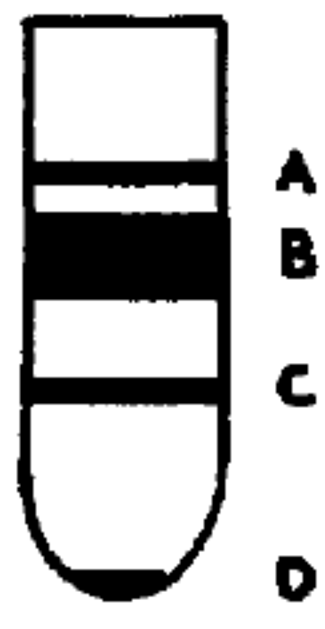
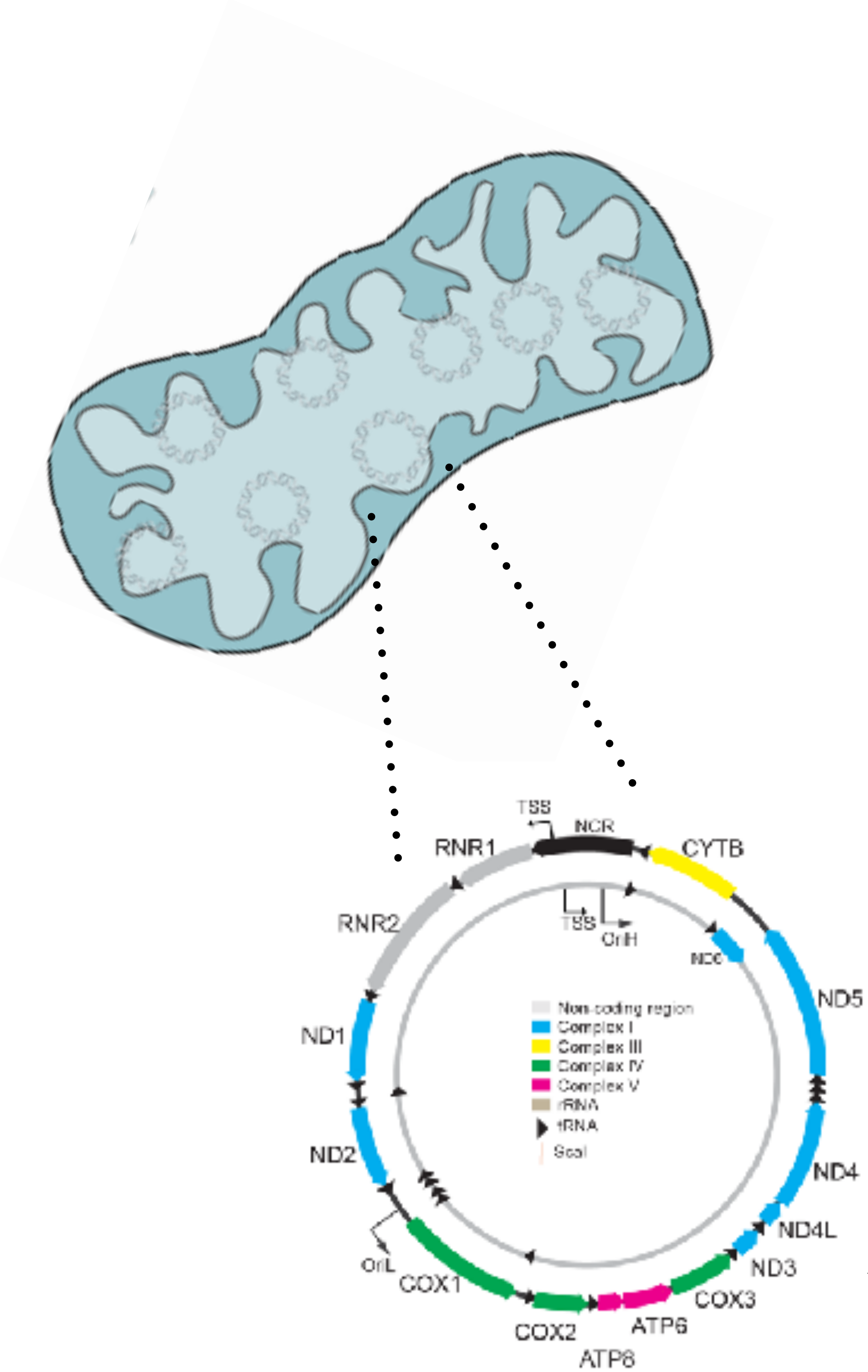
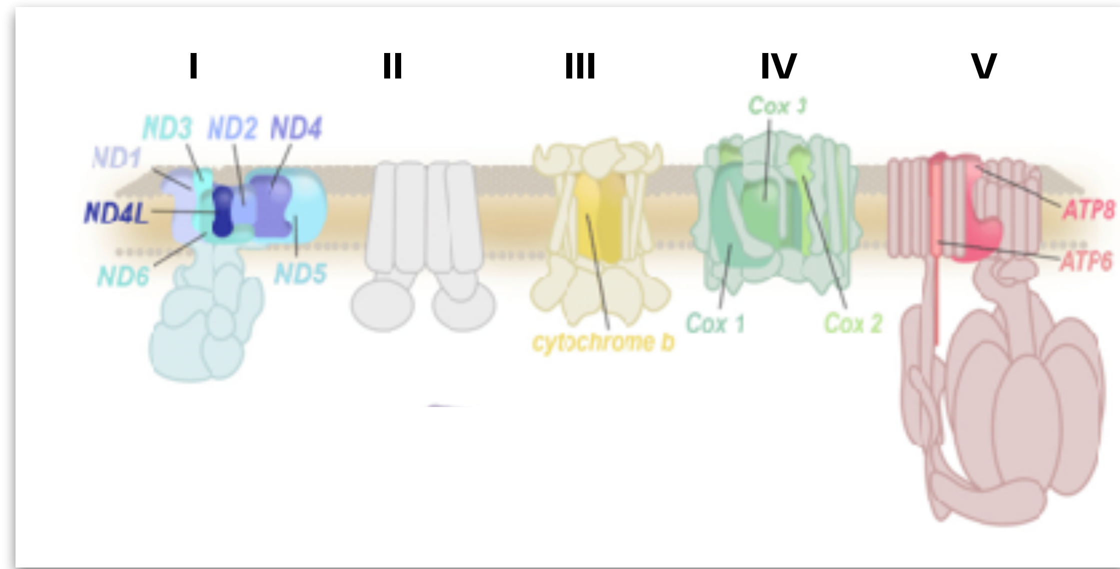


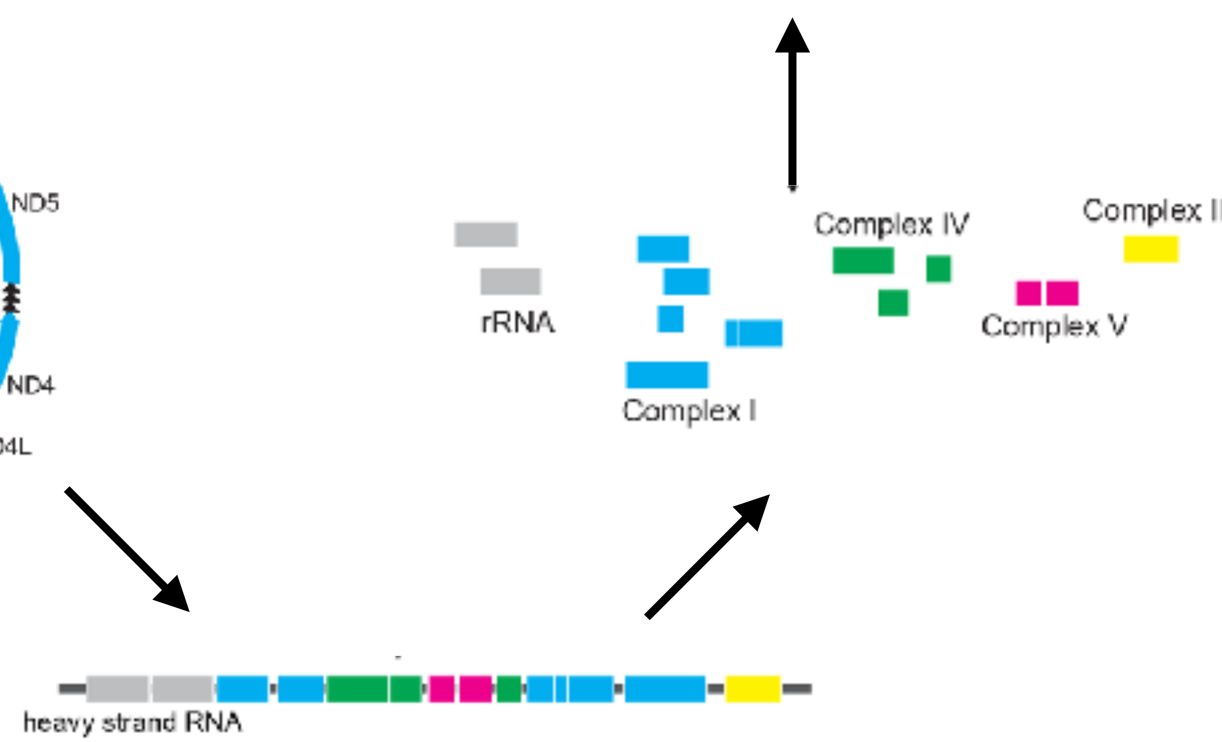
Fig. 4

Deoxyribose compounds precipitable by acid (DNA) µg / mg protein
1. 55
1. 78
1. 06 §)
1. 51
3. 60 +)
4. 30 &)
4. 30 &) +)

Mitochondrial genome; a 16Kb circular plasmid found in multiple copies

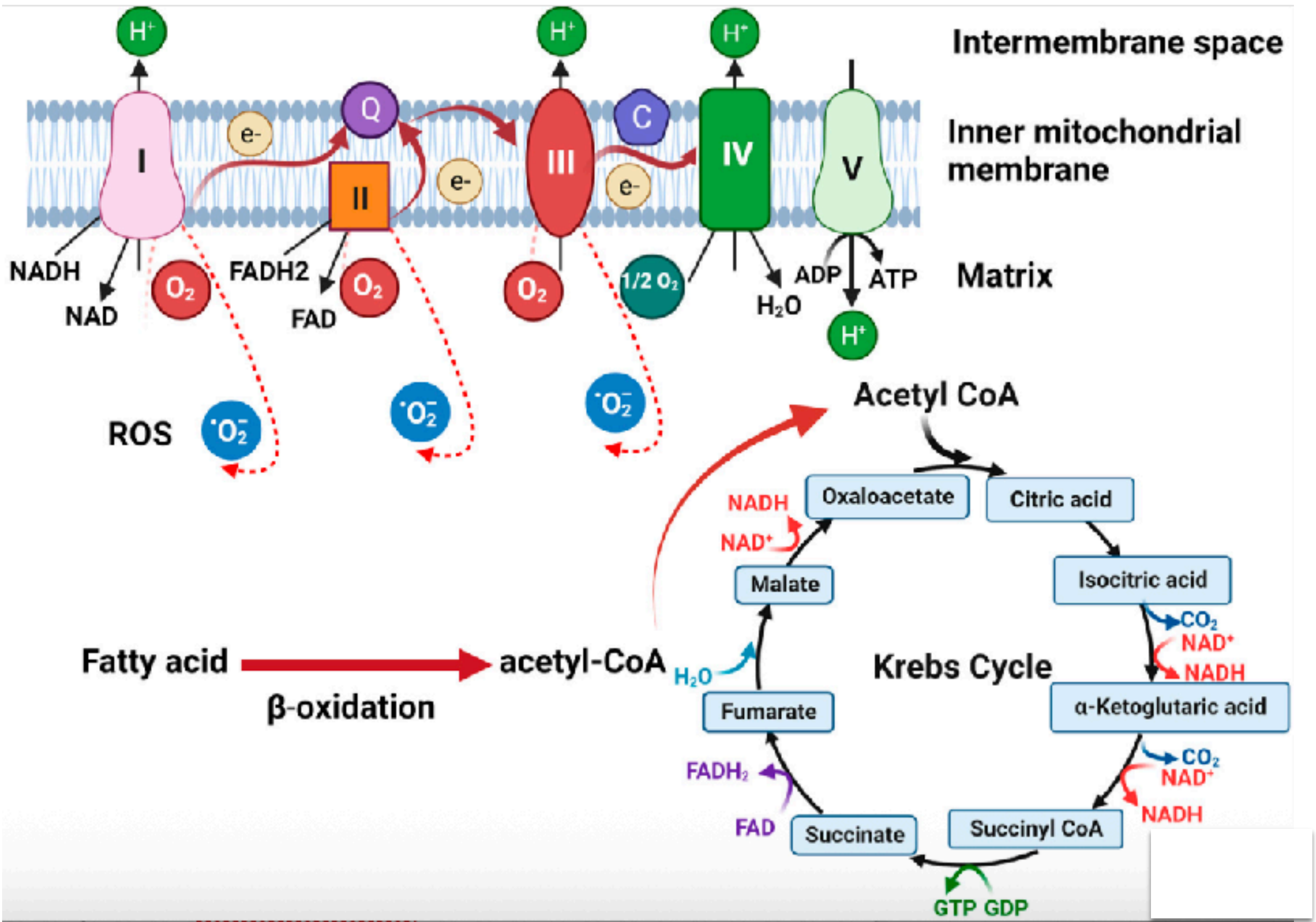




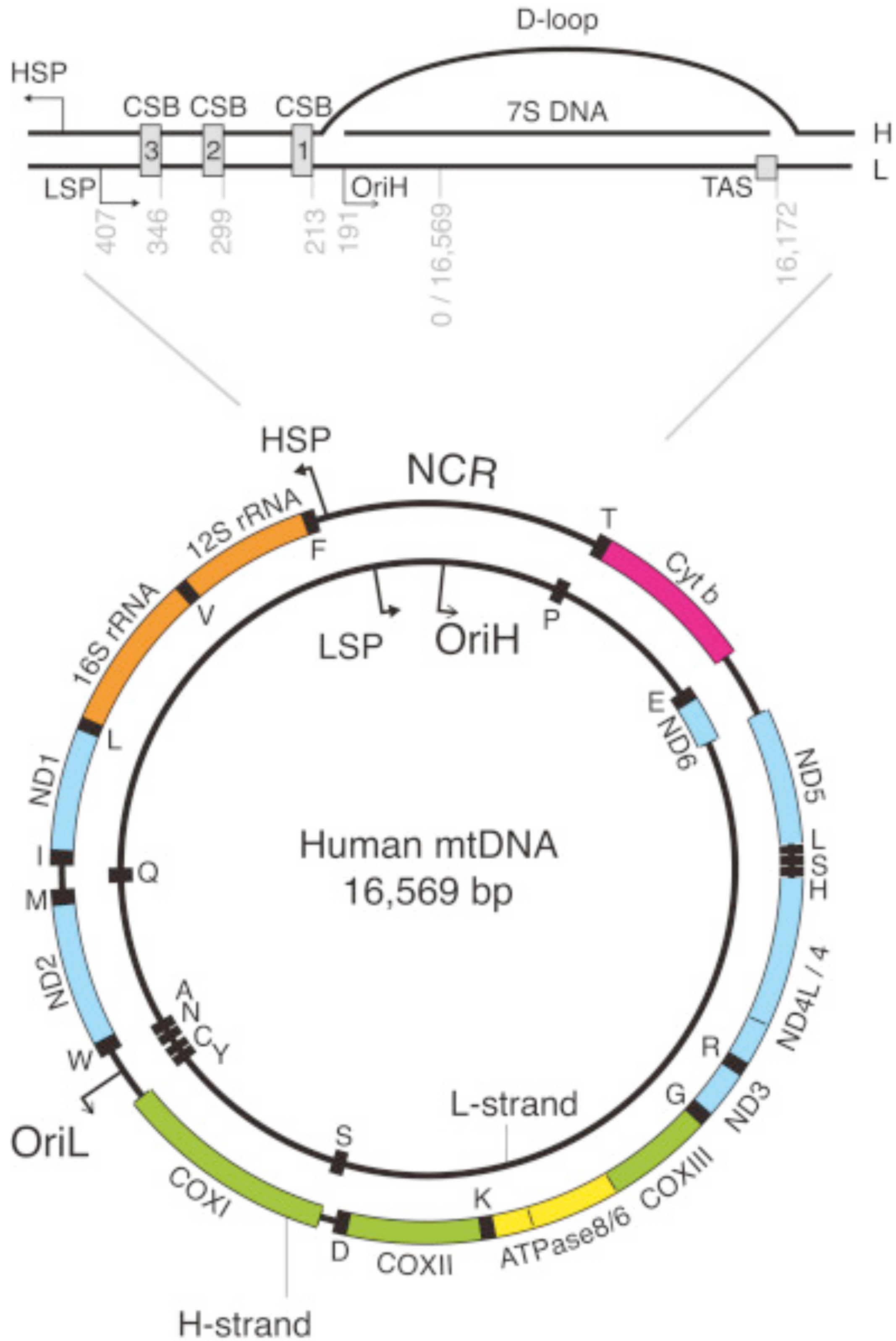


Hahn et al., 2019. PMID: 31514455

Mitochondrial Metabolism



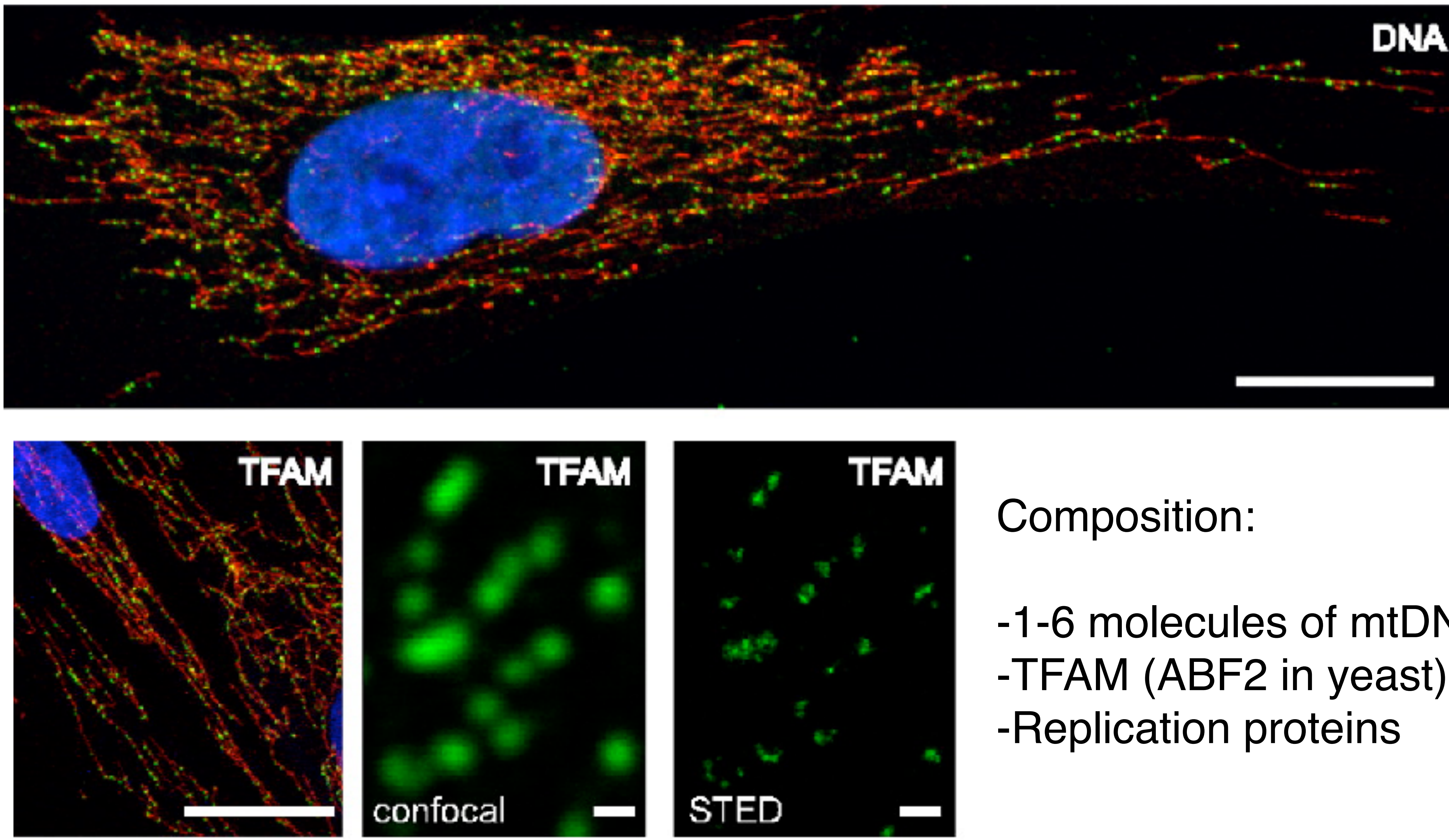
Organization and coding propertied of human mtDNA



- **16,659 bp** (*Homo sapiens*)
  - 37 coding genes
    - 22 tRNAs
    - 2 rRNAs
    - **13 peptides** (out of 1500 total)
- Two coding strands
  - **Heavy Strand** (higher G+T composition, less coding sequences)
  - **Light Strand** (lower G+T composition, more coding sequences)
- One conserved **Control region**, non coding: D-Loop, 1.1 Kbp
- **mtDNA is very gene dense** (no introns or intergenic sequences and polycistronic RNA
- **O_H and O_L are the two origins of replication** and separates the circle in a major and a minor arc.

Uhler and Falkenberg, 2015

nucleoids… the equivalence of nucleosomes

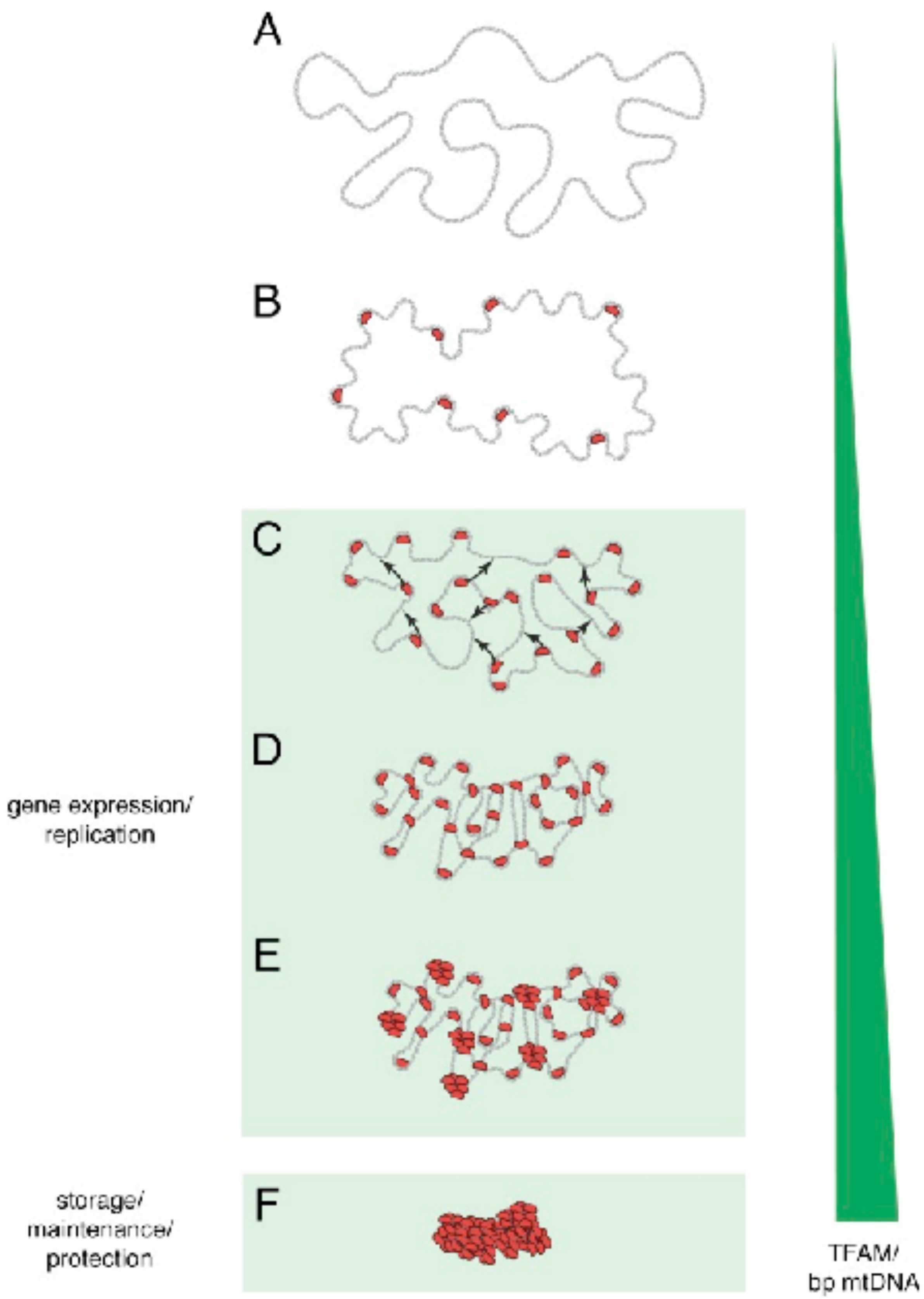


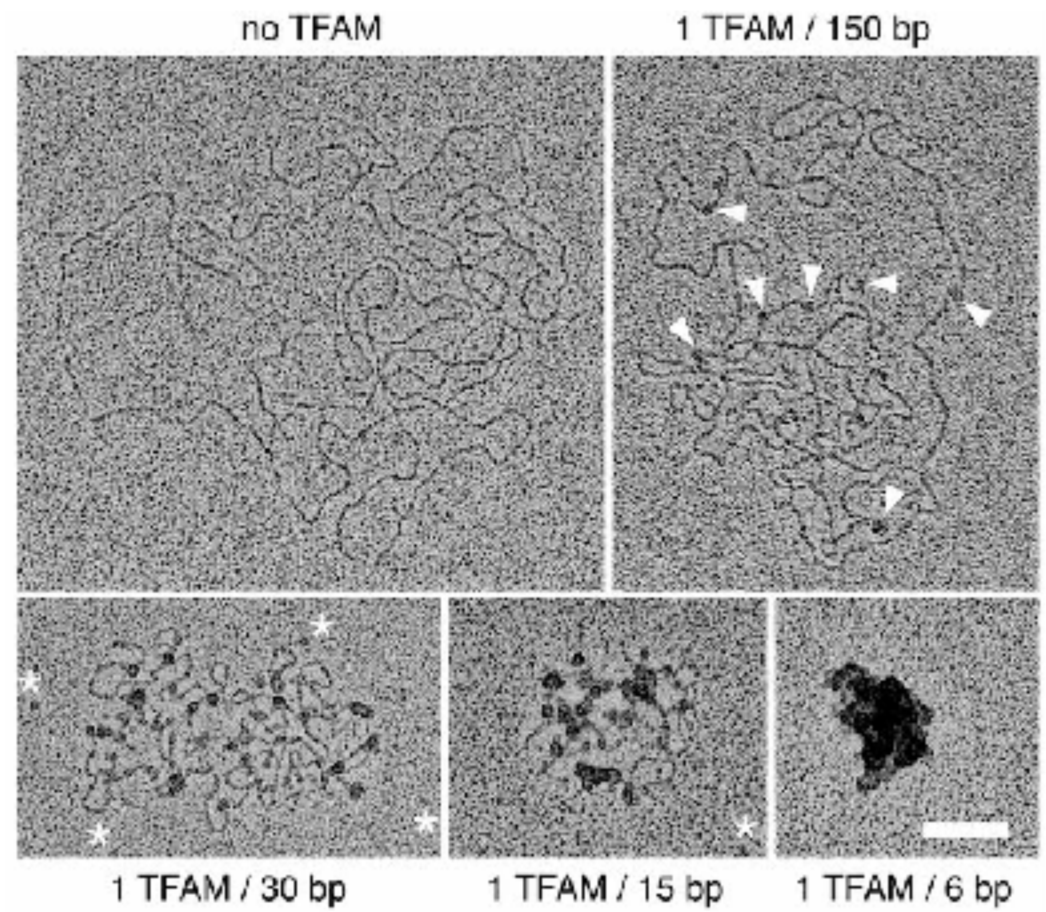
Composition:

-1-6 molecules of mtDNA
-TFAM (ABF2 in yeast)
-Replication proteins

Kukat et al., PNAS 2011

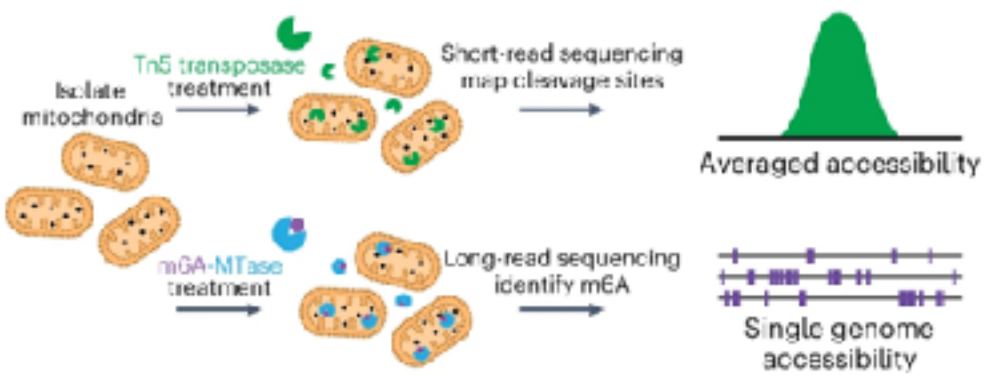
Model to explain mtDNA packaging into nucleoid

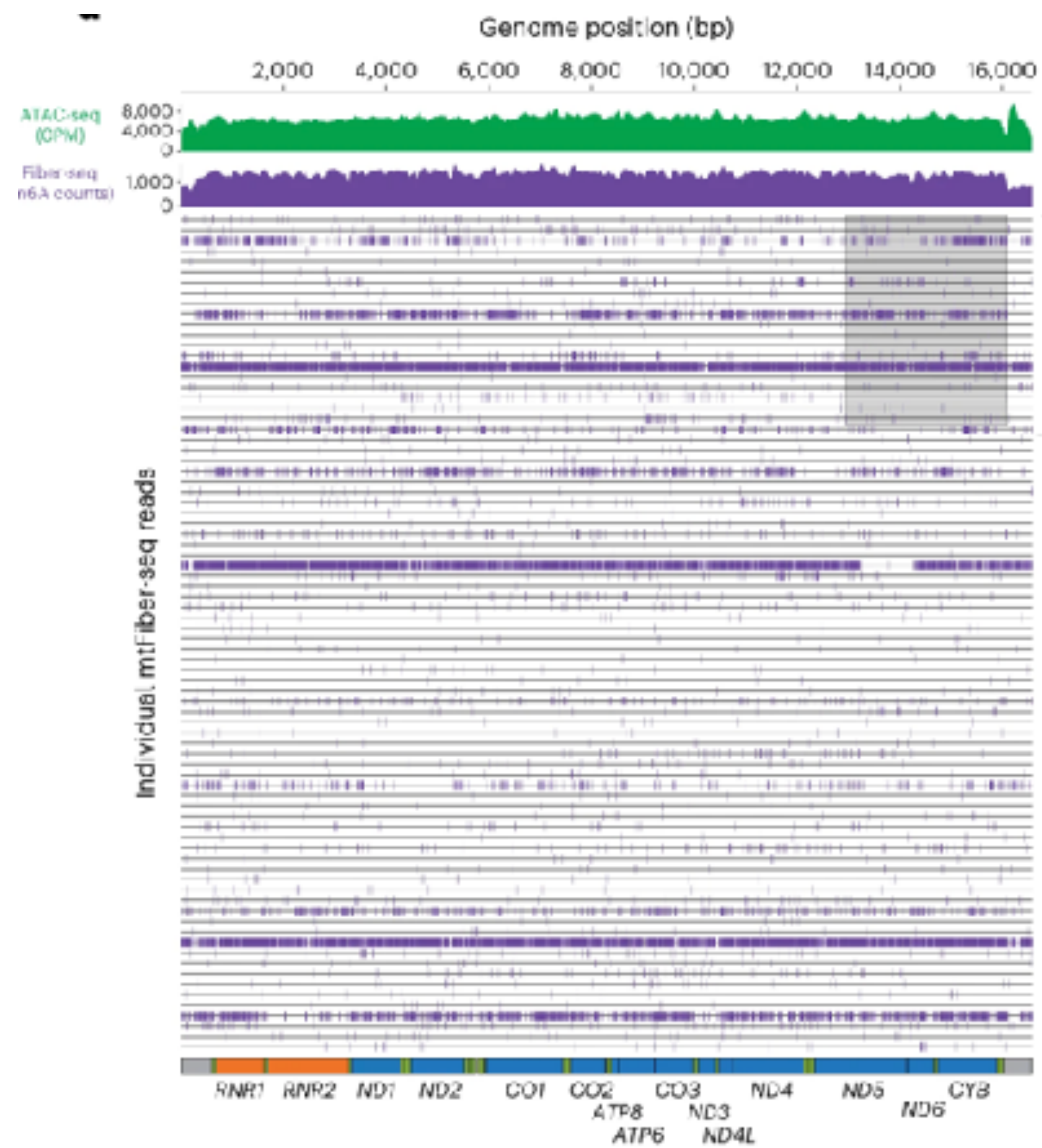


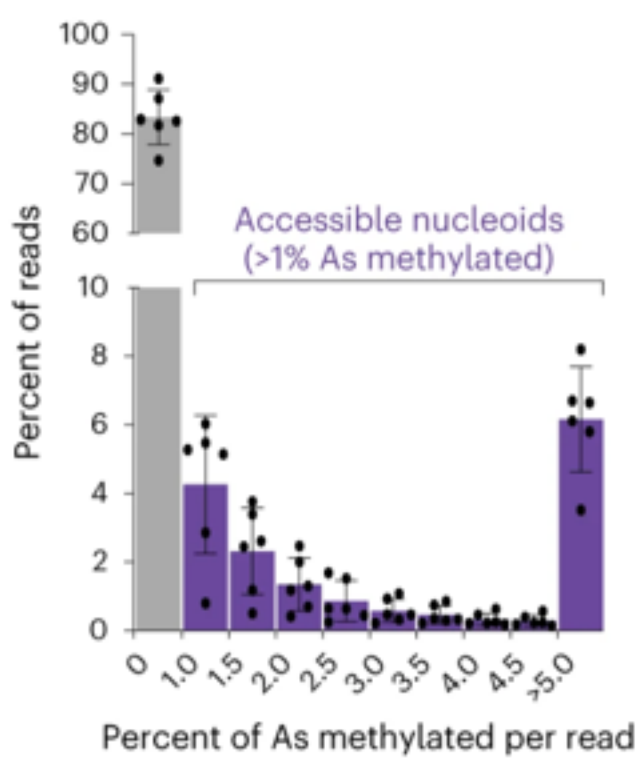


Kukat et al., PNAS 2015

FIBER-seq to monitor mtDNA accessibility







Isaac et al., 2024

Comparmentalization of replication, transcription, and translation

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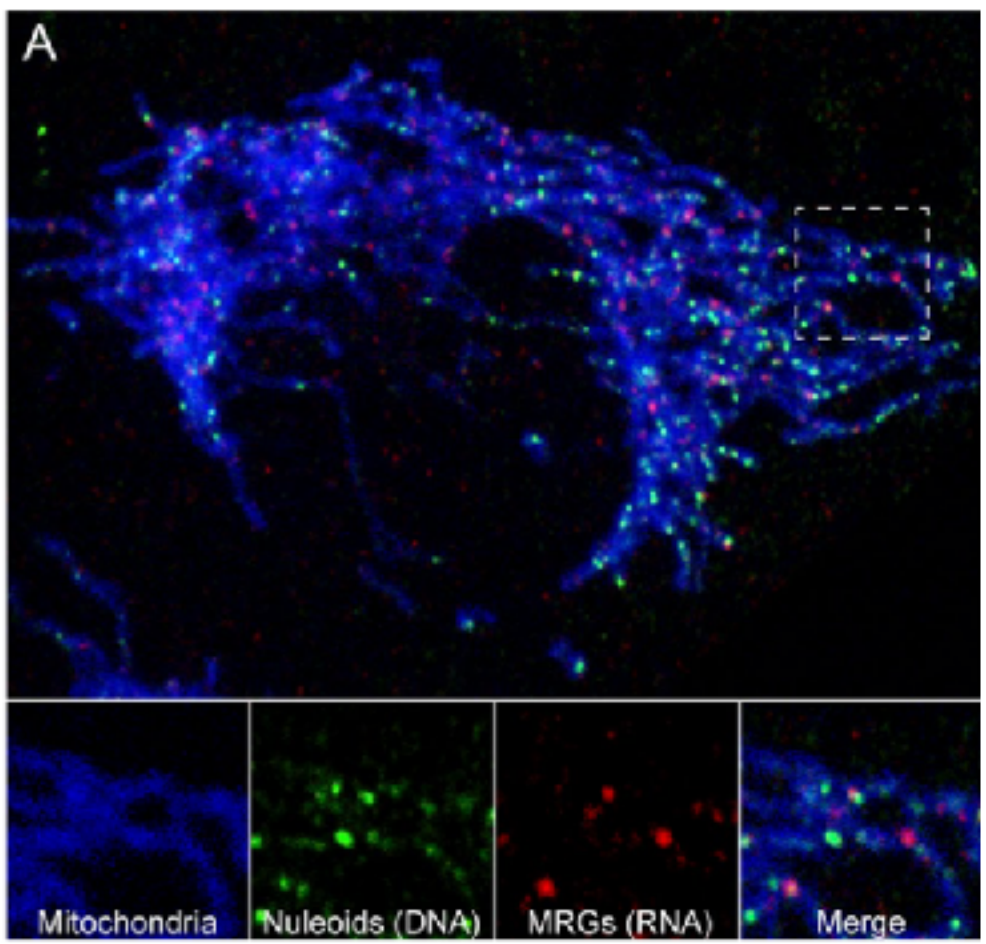
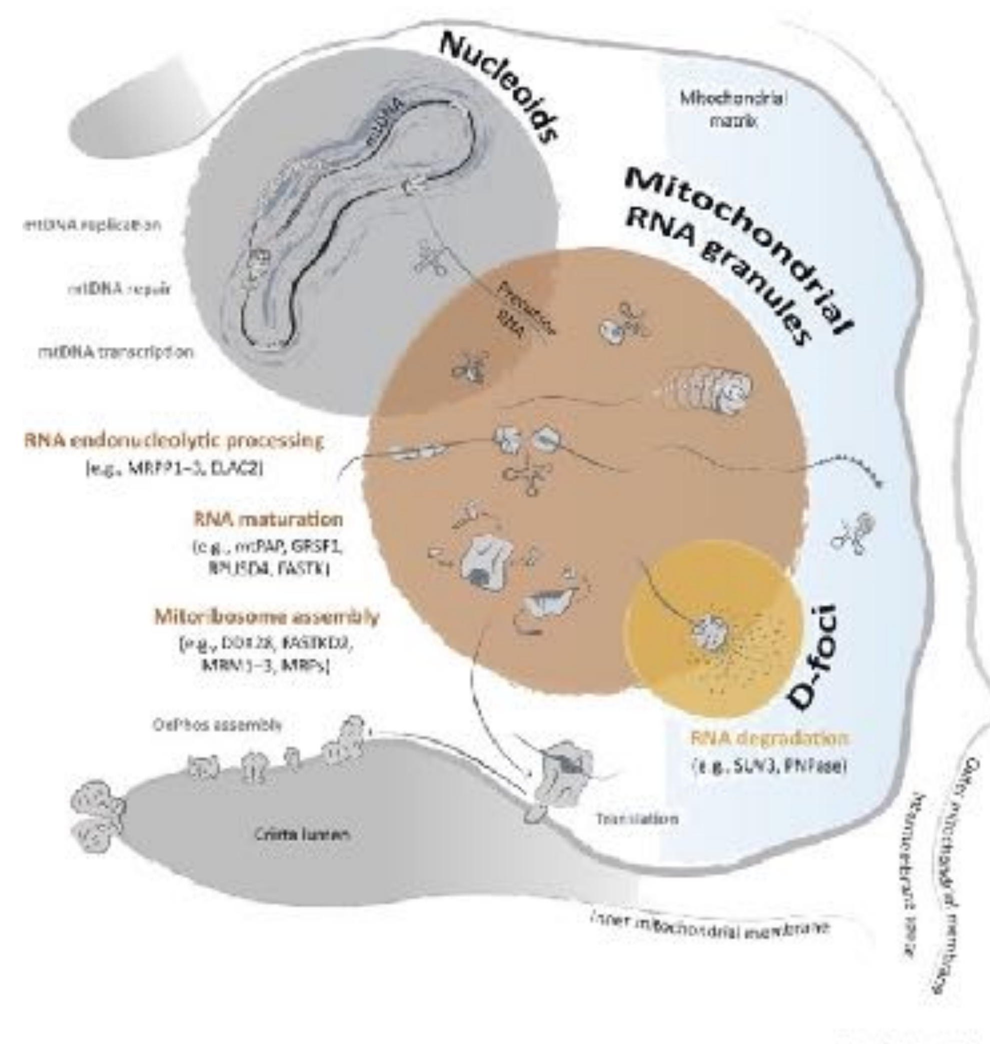
– Nucleoids: mtDNA replication, transcription and inheritance.
- RNA Granules (MRGs): RNA maturation and turnover are performed in distinct structures known as RNA granules (MRGs, in red in the figure).
- Translation: Mitoribosomes translate mature RNA at the inner membrane.

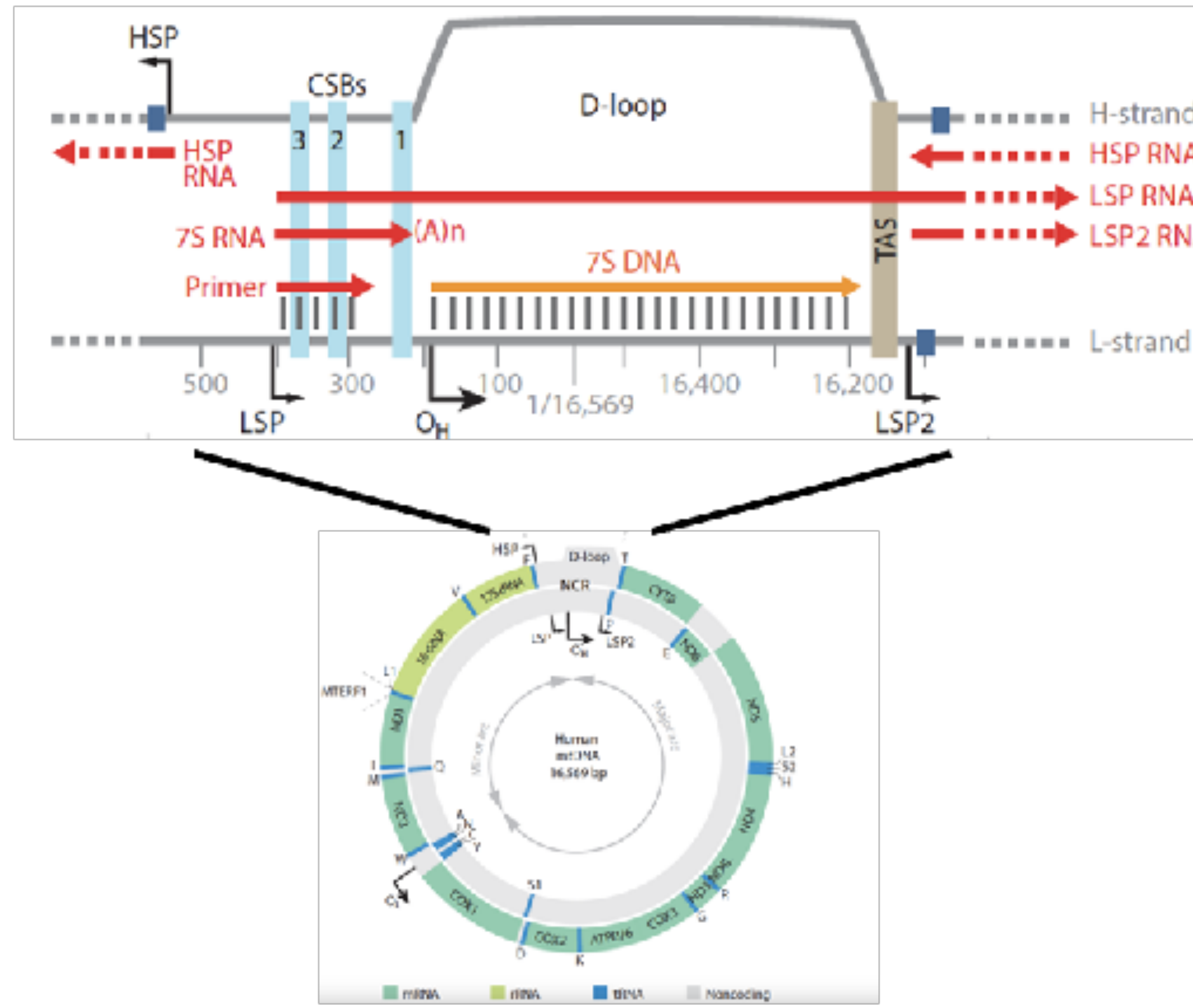




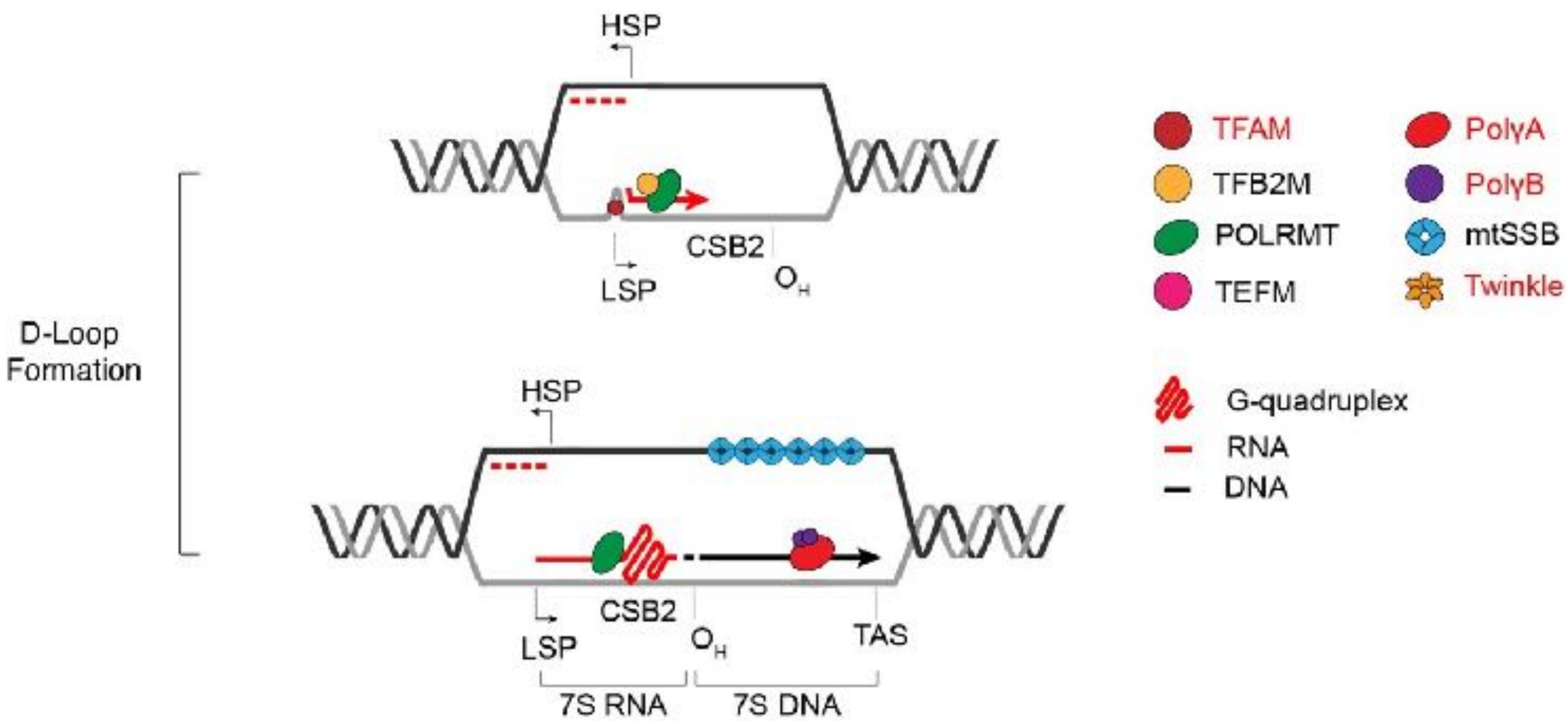
Pearce et al., 2017, Trends in Biochemical Sciences
Jourdain et al., 2016 JCB

The D-loop region: control of transcription and replication

- Replication and Transcription are mutually exclusive to avoid collisions of the two machineries.
- D-Loop (displacement loop): 1.1Kbp-long master regulatory sequence of mtDNA.
- Two promoter regions to transcribe the light and heavy strands (LSP1/2 and HSP) and three 3 conserved sequence blocks CSB1-3

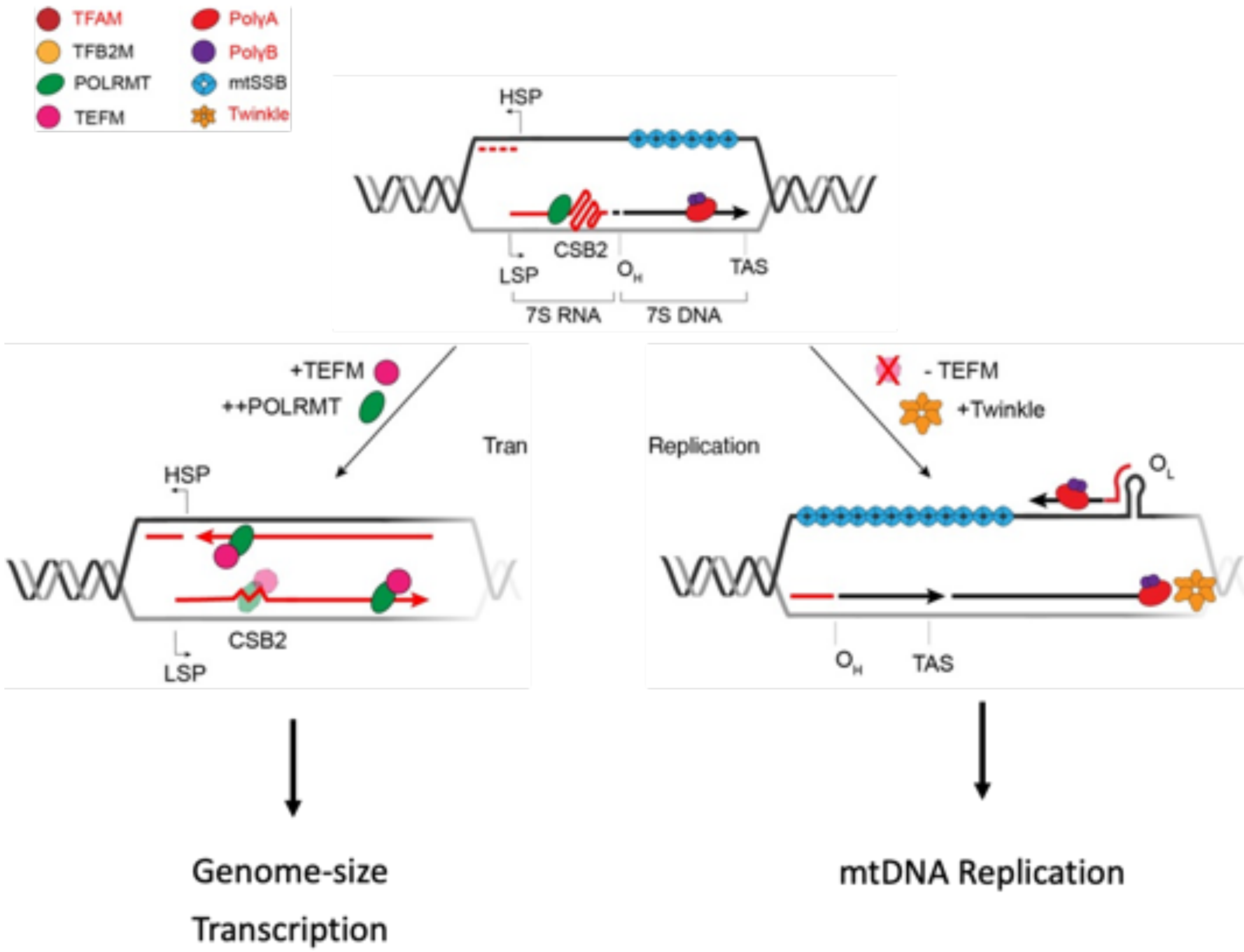


D-loop biogenesis



- TFAM bends mtDNA upstream of LSP.
- POLRMT and TFB2M are recruited at the LSP and initiate low-processive RNA synthesis.
- Transcription "stalls" at CSB2, just before O_H due to G-quadruplex structures. This RNA molecule is known as 7S-RNA
- POLγ uses 7S-RNA as a primer to synthetize a short DNA molecule (7S-DNA)
- 7S-DNA synthesis is terminated at the TAS sequence through an unknown molecular process. POLG does not automatically proceeds with genome-size replication.

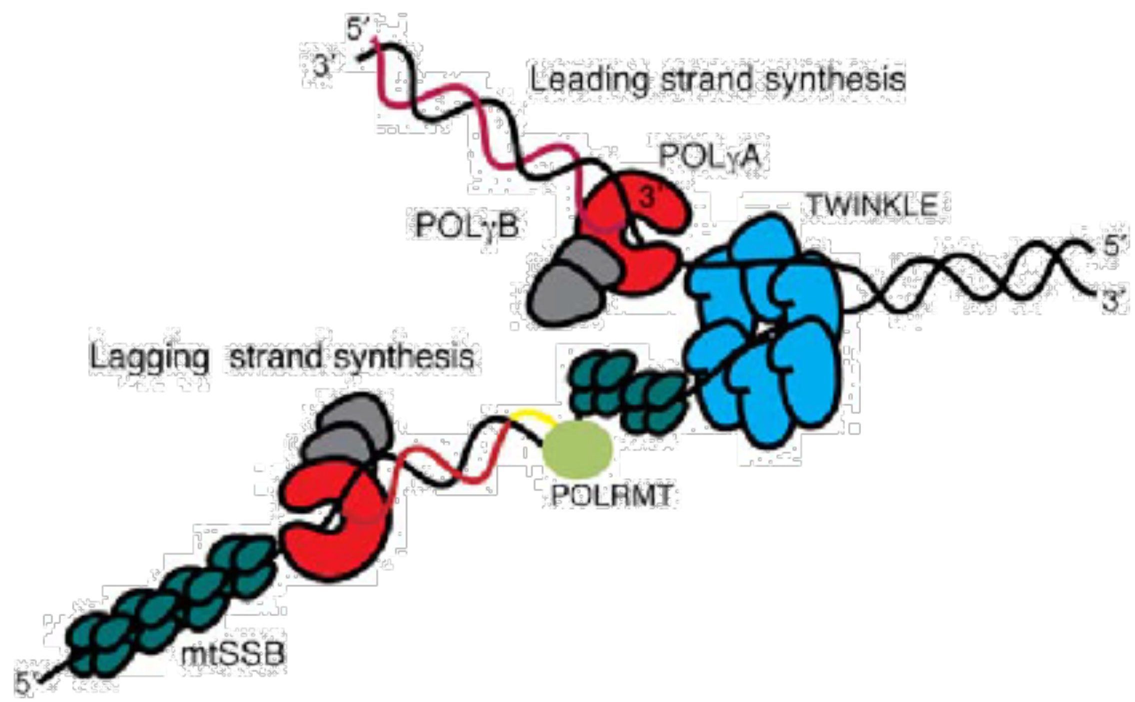
Replication-transcription switch



- POLRMT is not processive enough to melt the G-quadruplex.
- The switch is operated by TEFM taking the place of TFB2M
- If TEFM is loaded, POLRMT proceeds over the G-quadruplex and complete transcription of the entire genome.
- If TEFM is not loaded, the 7S-RNA is used by POLγ to generate the 7S-DNA.
- Loading of the helicase Twinkle at the TAS will fully ”fire” the O_H committing the 7S-DNA to heavy strand replication.
-

Agaronyan K, Morozov Y, Anikin M and **Temiakov D**, Science, 2015.

mtDNA replication: The Essential Machinery



Minimal Mitochondrial Replisome reconstituted in vitro and fully functional:

- **POLγ** A and **POLγ** B (polymerase subunits)
- **Twinkle** (hexameric helicase)
- **mtSSB** (single stranded DNA binding protein)
- **POLRMT** (RNA polymerase)

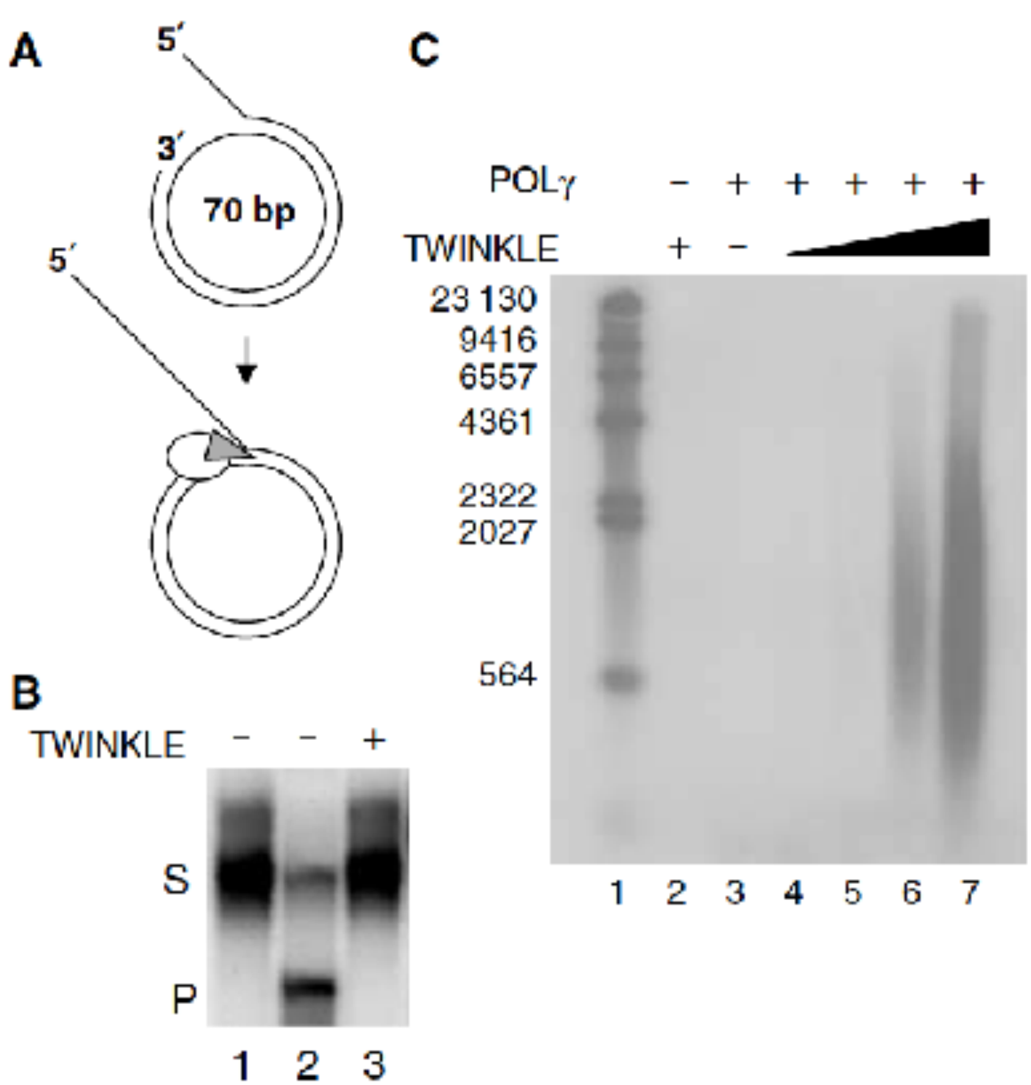
Korhonen J, Hoi Pham X, Pellegrini M, Falkenberg M, EMBO J, 2004

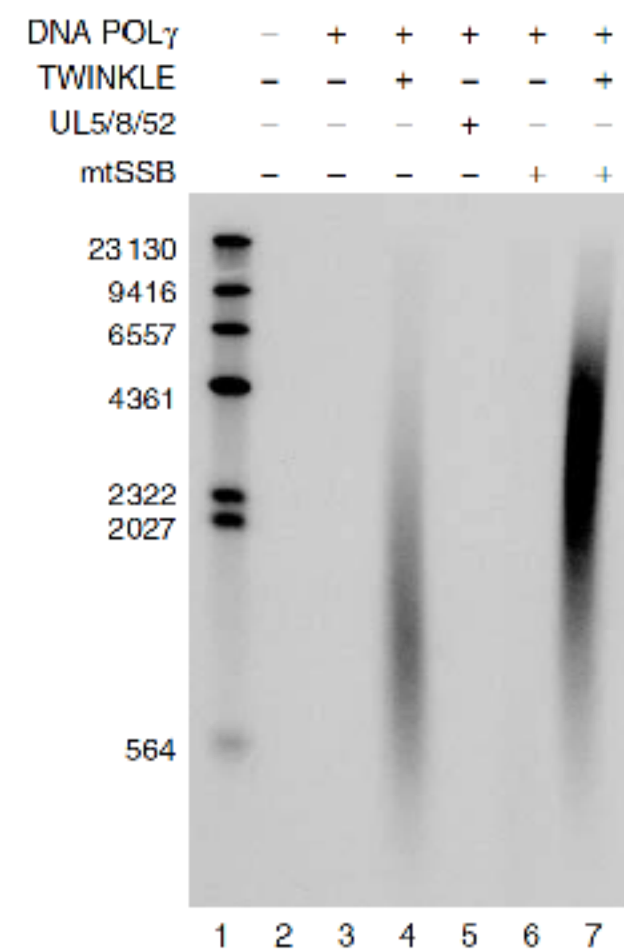
mtDNA replication: minimal replisome

Reconstitution of a minimal mtDNA replisome *in vitro*

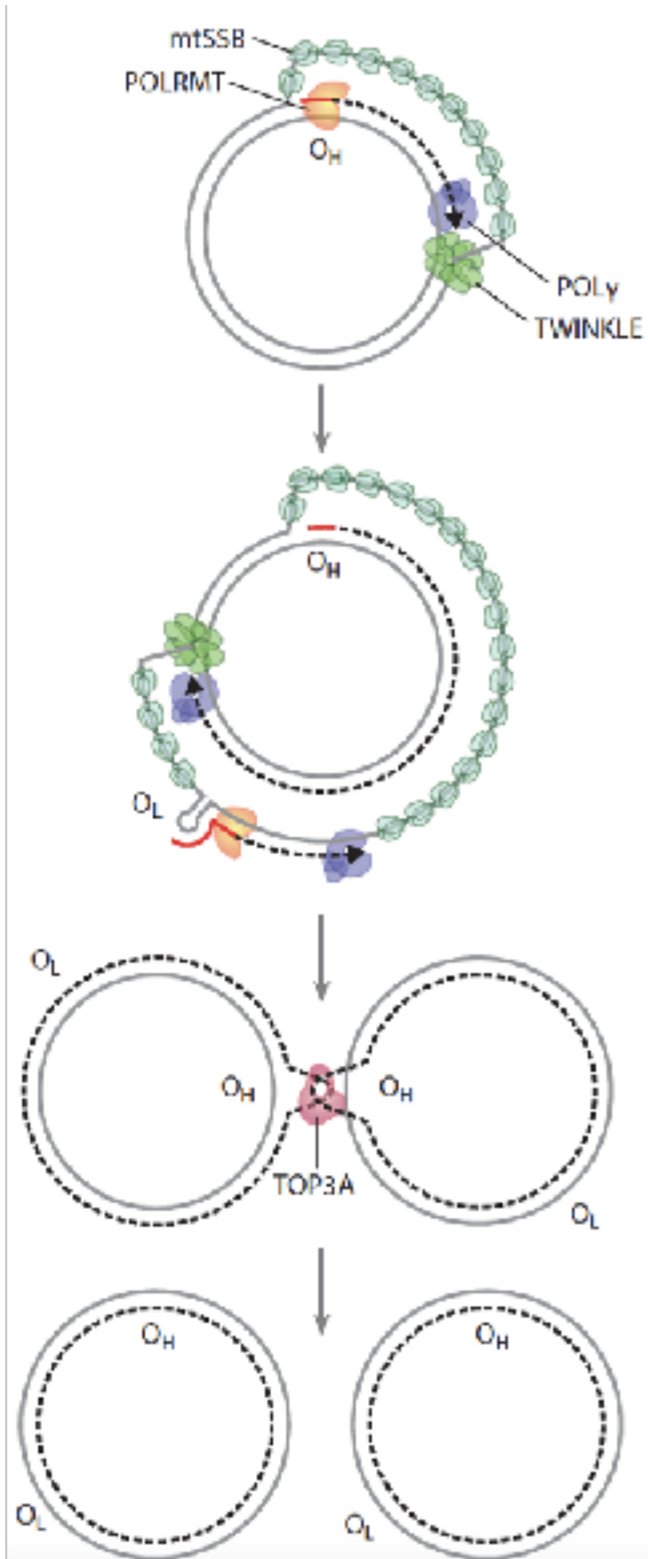
Jenny A Korhonen, Xuan Hoi Pham,
Mina Pellegrini and Maria Falkenberg*

The EMBO Journal (2004) 23, 2423–2429





Strand displacement model of DNA replication



1. O_H Firing for Leading Strand Synthesis performed by POLγ assisted by the helicase Twinkle.

2. Lagging Strand is coated with mtSSB to protect from damage and recombination.

3. O_L is exposed and forms a hairpin structure that recruits POLRMT to synthetize a short RNA primer used for the replication of the lagging strand.

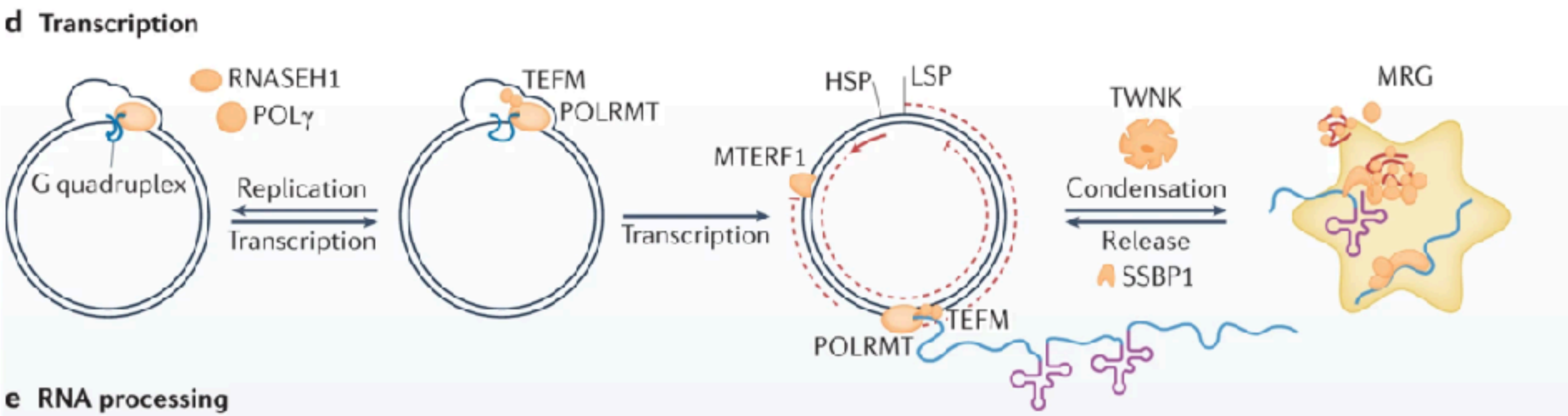
4. RNA Primers and flaps are processed at the end by RNaseH1 (long primers), FEN1 (short primers) or MGME1 (O_H primer).

5. Ligation is performed by Ligase3.

6. Decatenation is performed by Top3A.

Falkenberg Maria, Larsson Nils-Göran, Gustafsson Claes M., Annual Review of Biochemistry, 2024

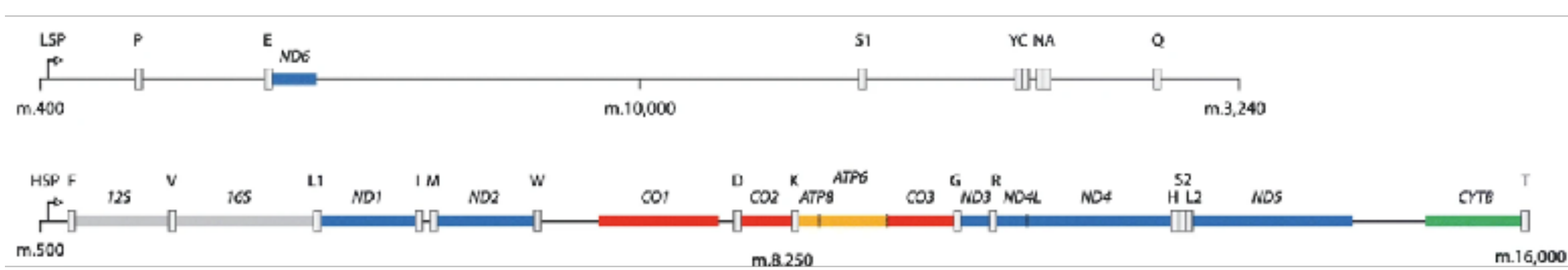
mtDNA transcription and mtRNA maturation



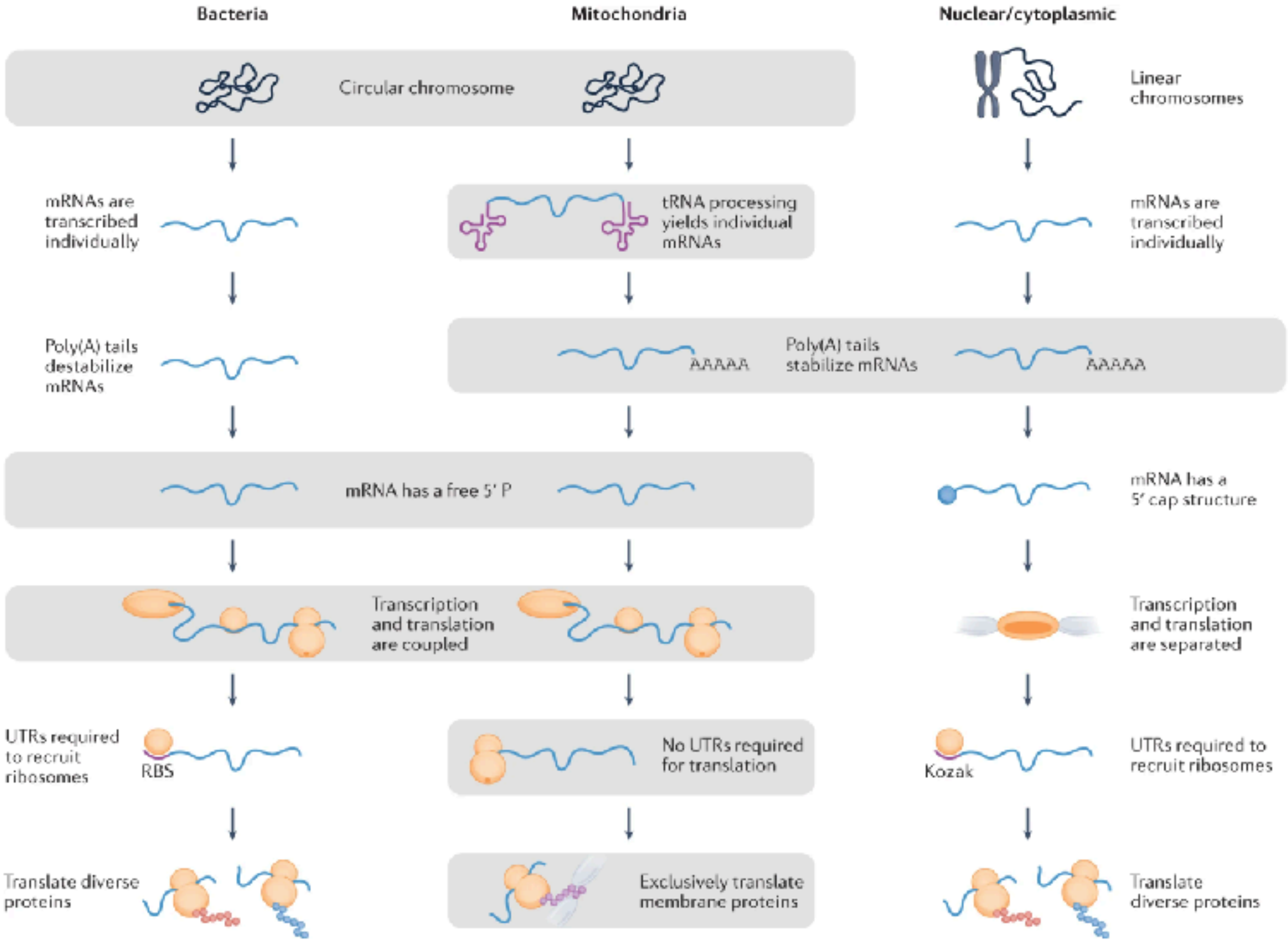
HSP1 Transcript
2 tRNAs
2 rRNAs

HSP2 Transcript
10 mRNAs (12 proteins)
14 tRNAs
2 rRNAs

LSP Transcript
1 mRNA (1 protein)
8 tRNAs

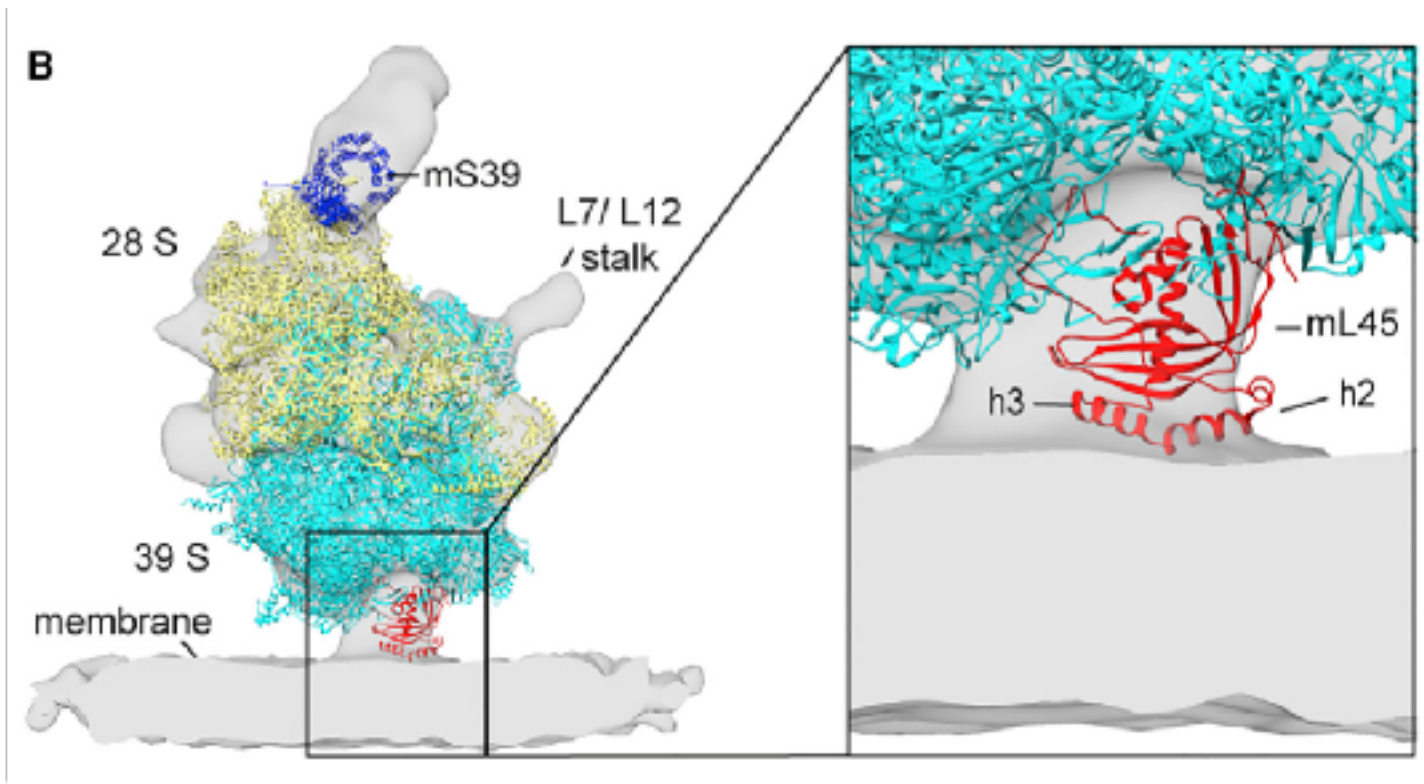


Compare and contrast with bacterial and nuclear genome organization



Translation: the Mitoribosome

Mitochondrial ribosomal proteins are provided by the nucleus while the RNA components are encoded in the mtDNA.



39S large subunit: 52 proteins + 16S-rRNA
28S small subunit: 30 proteins + 12S-rRNA

On the inner membrane of the mitochondria.

Translation co-occurs with membrane insertion.

Only membrane proteins are translated in the mitochondria.

Robert Englmeier, Stefan Pfeffer, Friedrich Förster, Science 2017

Unique genetic code

- It can be translated by just 22 tRNAs (instead of 30) thanks to extreme “wobbling”.
- AGA and AGG are interpreted as STOP codons in mitochondria, not arginine.
- AUA is used to code for Methionine, not Isoleucine (but not as start codon).
- UGA is not a stop codon, but codes for Tryptophane.

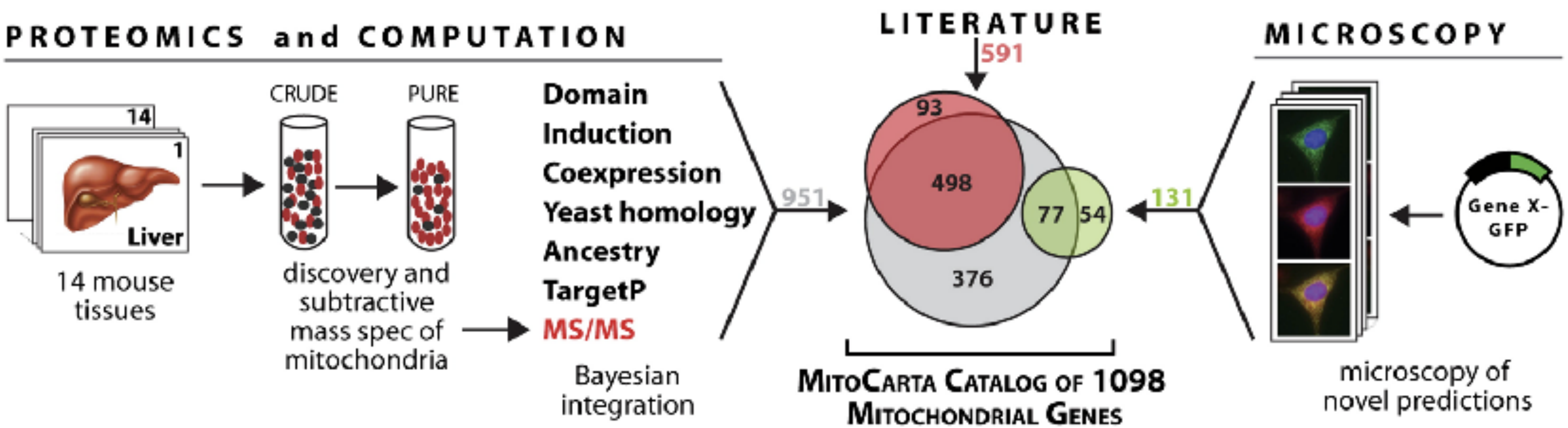
Cod	Universal	Human
UGA	STOP	Trp
AGA	Arg	STOP
AGG	Arg	STOP
AUA	Ile	Met

Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC	UCC	UAC	UGC	C
	UUA Leu	UCA	UAA Stop	UGA Trp	A
	UUG	UCG	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC	ACC	AAC	AGC	C
	AUA Met	ACA	AAA Lys	AGA Stop	A
	AUG	ACG	AAG	AGG Stop	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glu	GGA	A
	GUG	GCG	GAG	GGG	G

Mitocarta: An Inventory of Mitochondrial Genes

~1100 Proteins imported from the Nucleus

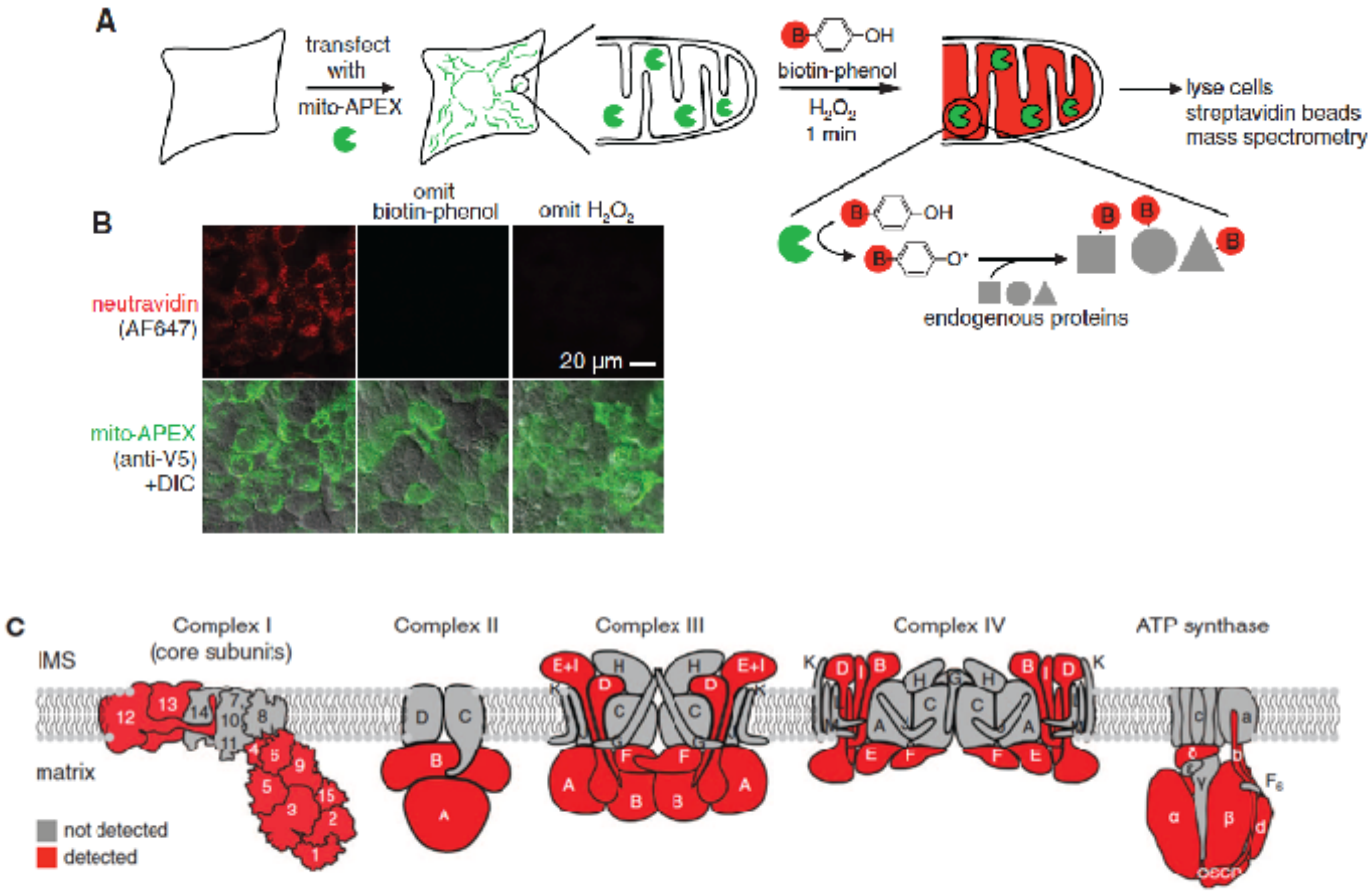


http://www.broadinstitute.org/scientific-community/science/programs/metabolic-disease-program/publications/mitocarta/mitocarta-in-0

Pagliarini et al., Cell. 2008

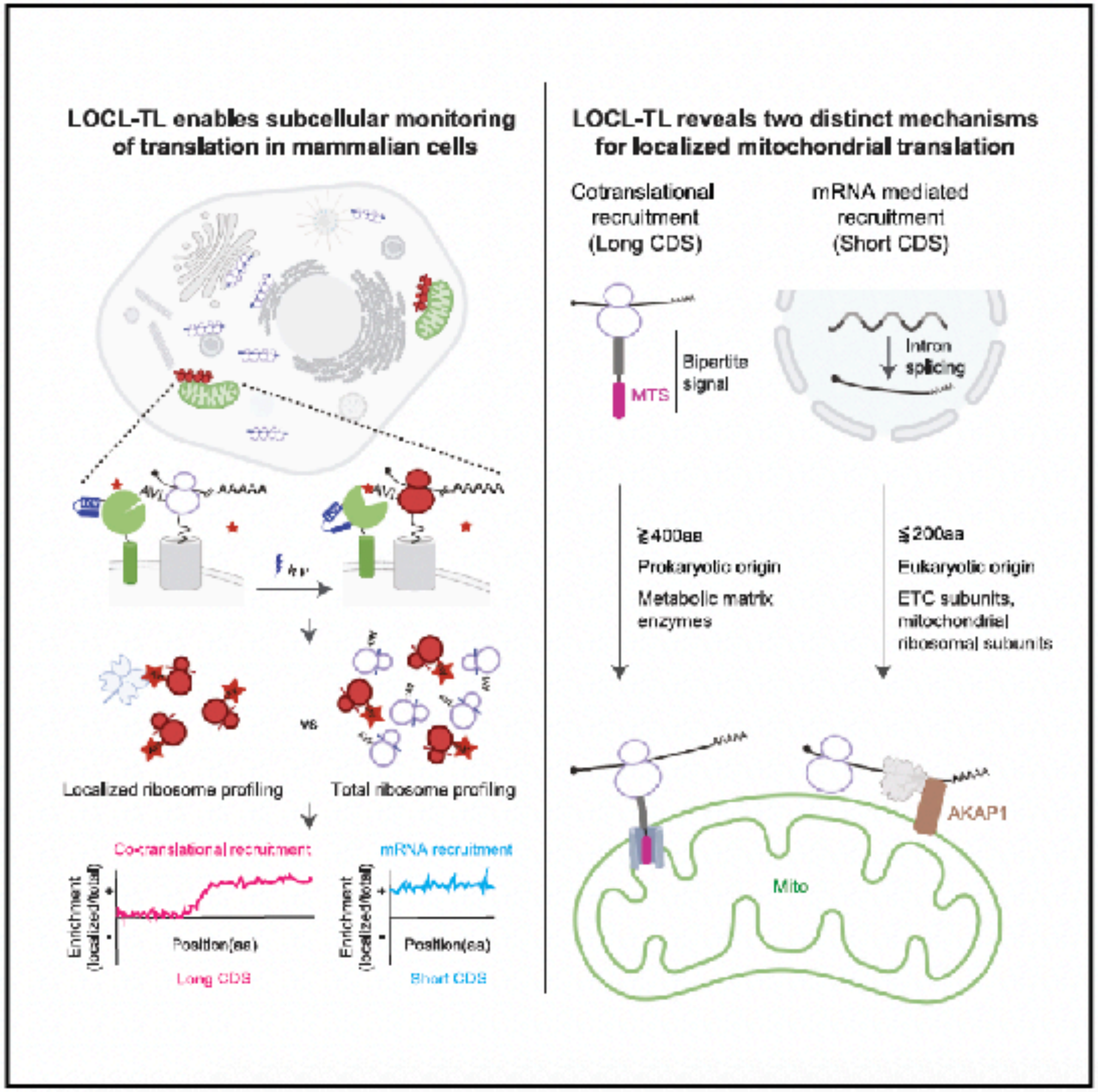
Proteomic approach to place proteins within the mitochondrial matrix

Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging
Hyun-Woo Rhee *et al.*
Science **339**, 1328 (2013);
DOI: 10.1126/science.1230593



Rhee et al., Science. 2013

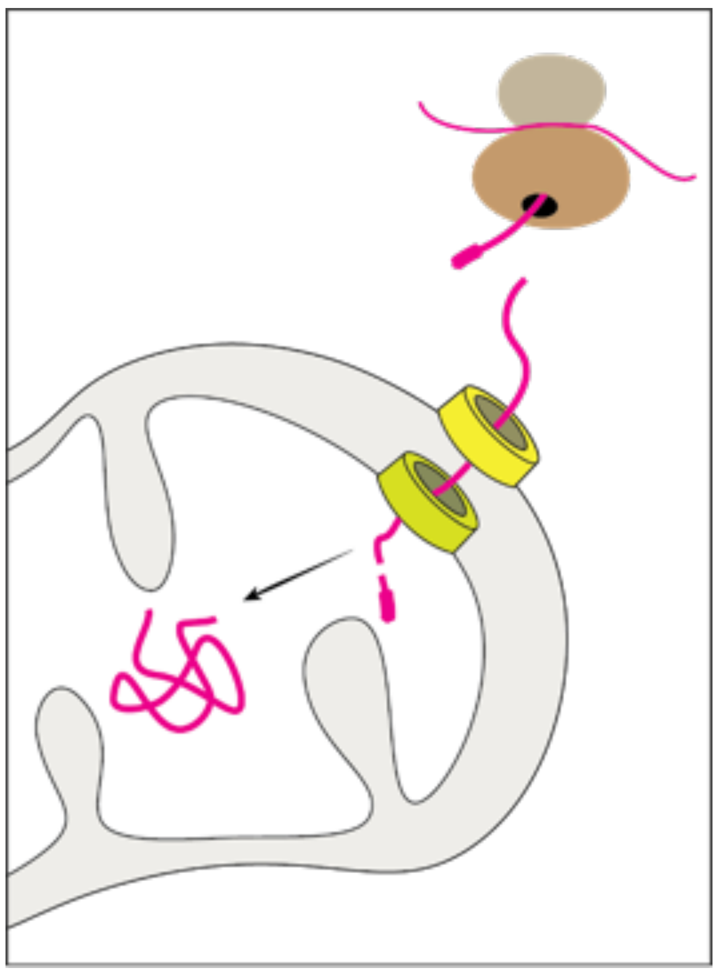
The logic of localized mitochondrial translation



- An optogenetic approach enables monitoring of localized translation in mammalian cells
- Mitochondrially localized translation is mediated by two distinct strategies
- A bipartite signal sequence enables cotranslational translocation of long CDSes
- AKAP1 targets short CDS transcripts to mitochondria independent of translation

Zhu et al., Cell 2025

Mitochondrial protein import senses mitochondrial stress

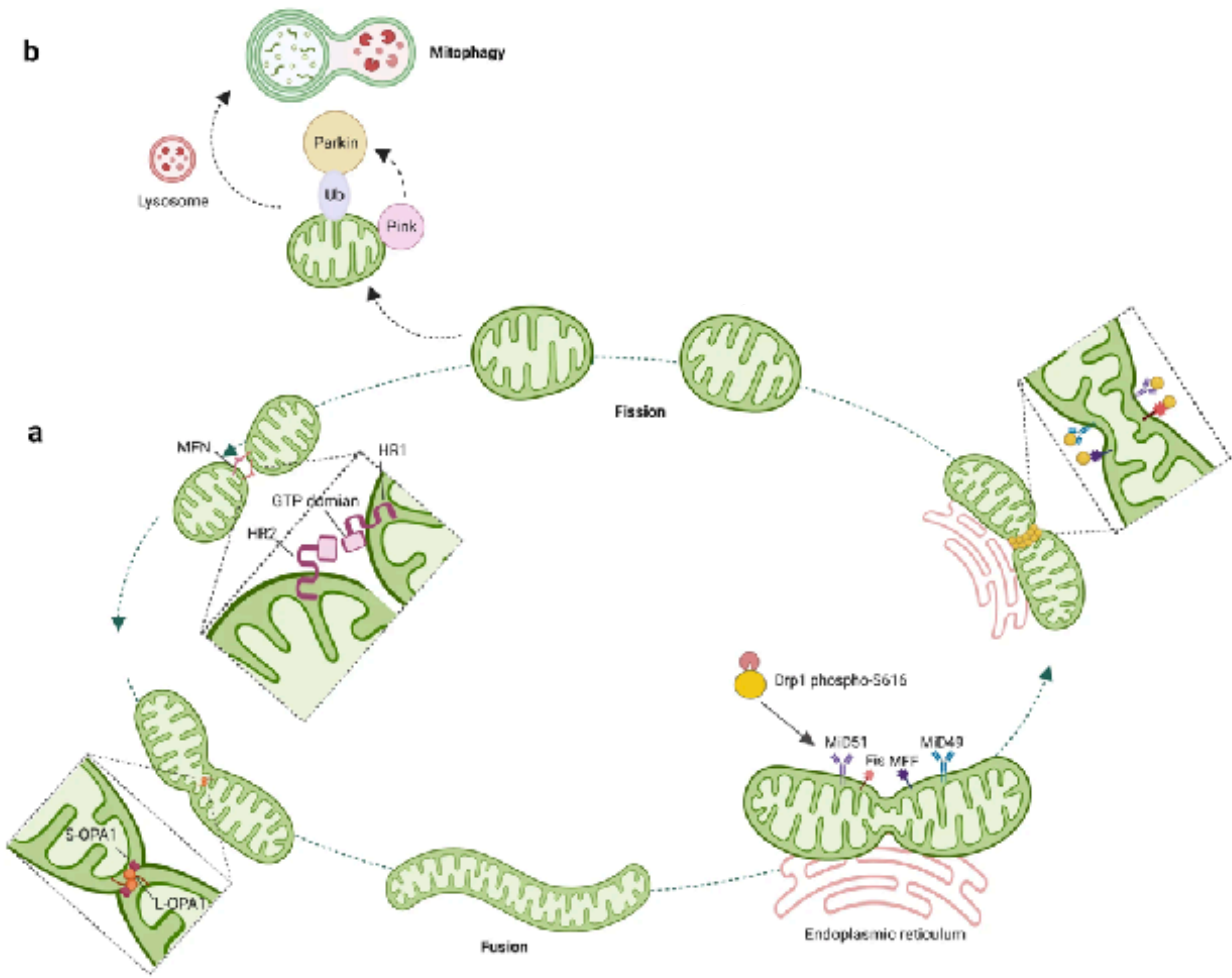


<u>Stress</u>
•Decreased membrane potential
•Decreased ATP
•Overloading with precursors
•Proteostress from mitochondria and cytosol

<u>Quality control mechanisms</u>
•mitoTAD, mitoCPR, mPOS, UPRam
•Mitophagy
UPRmt

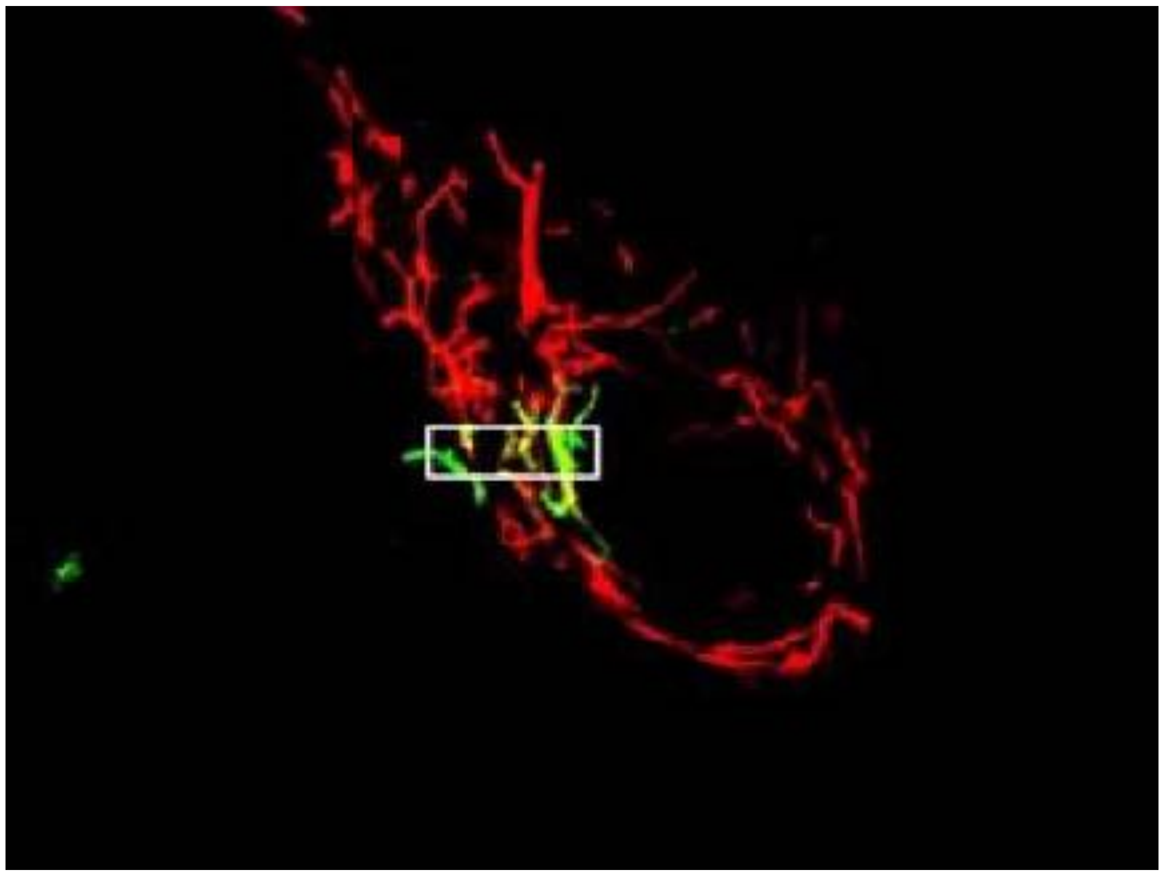
Wang, Chen. 2015. PMID: 26192197
Wrobel, et al. 2015. PMID: 26245374
Weidberg, Amon. 2018. PMID: 29650645
Sekine, Youle. 2018 PMID: 29325568
Mårtensson, et al. 2019. PMID: 31118508
Shpilka et al. 2021. PMID: 33473112

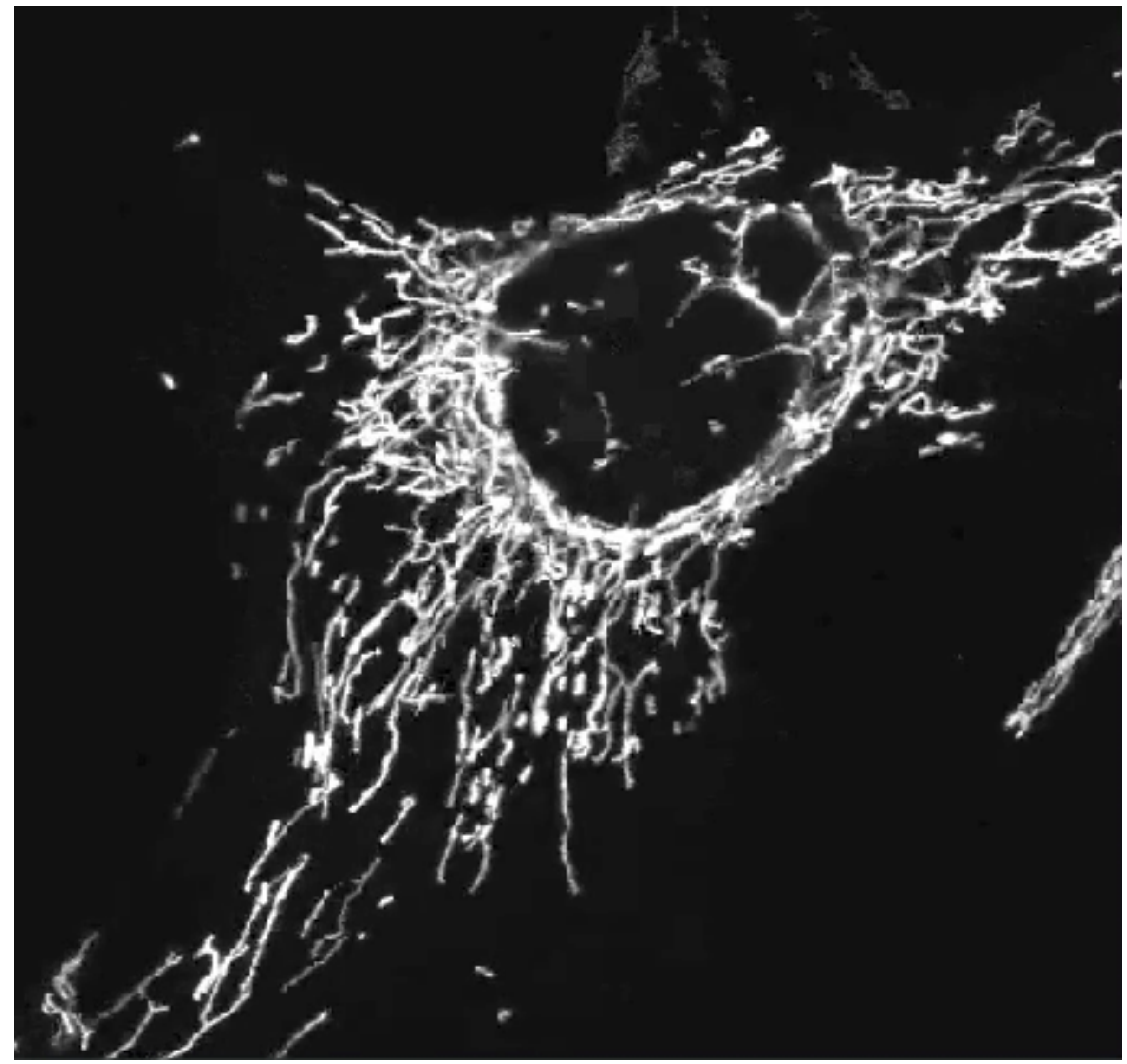
Mitochondrial dynamics: fission, fusion and mitophagy



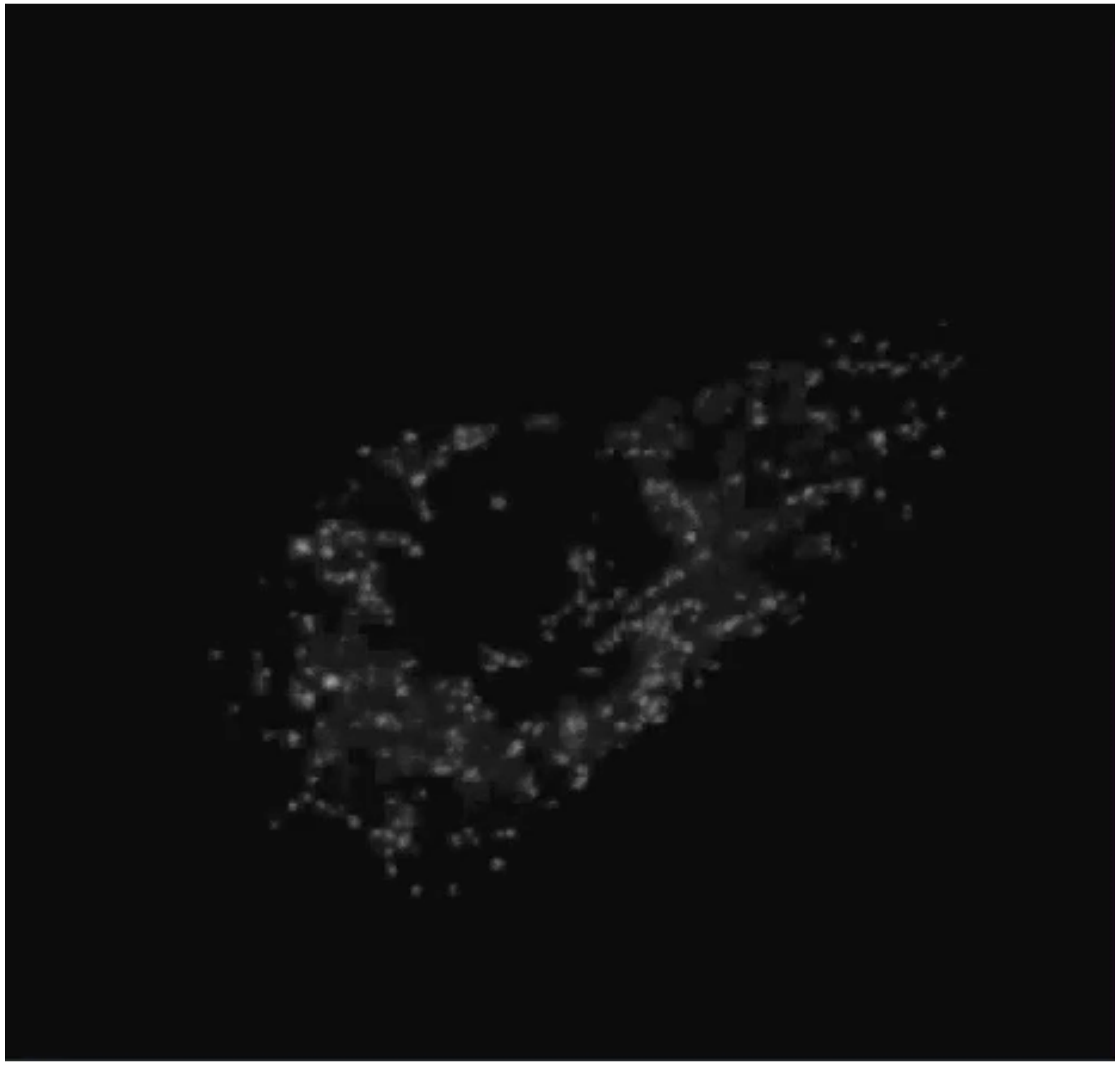
Chen et al. , 2023

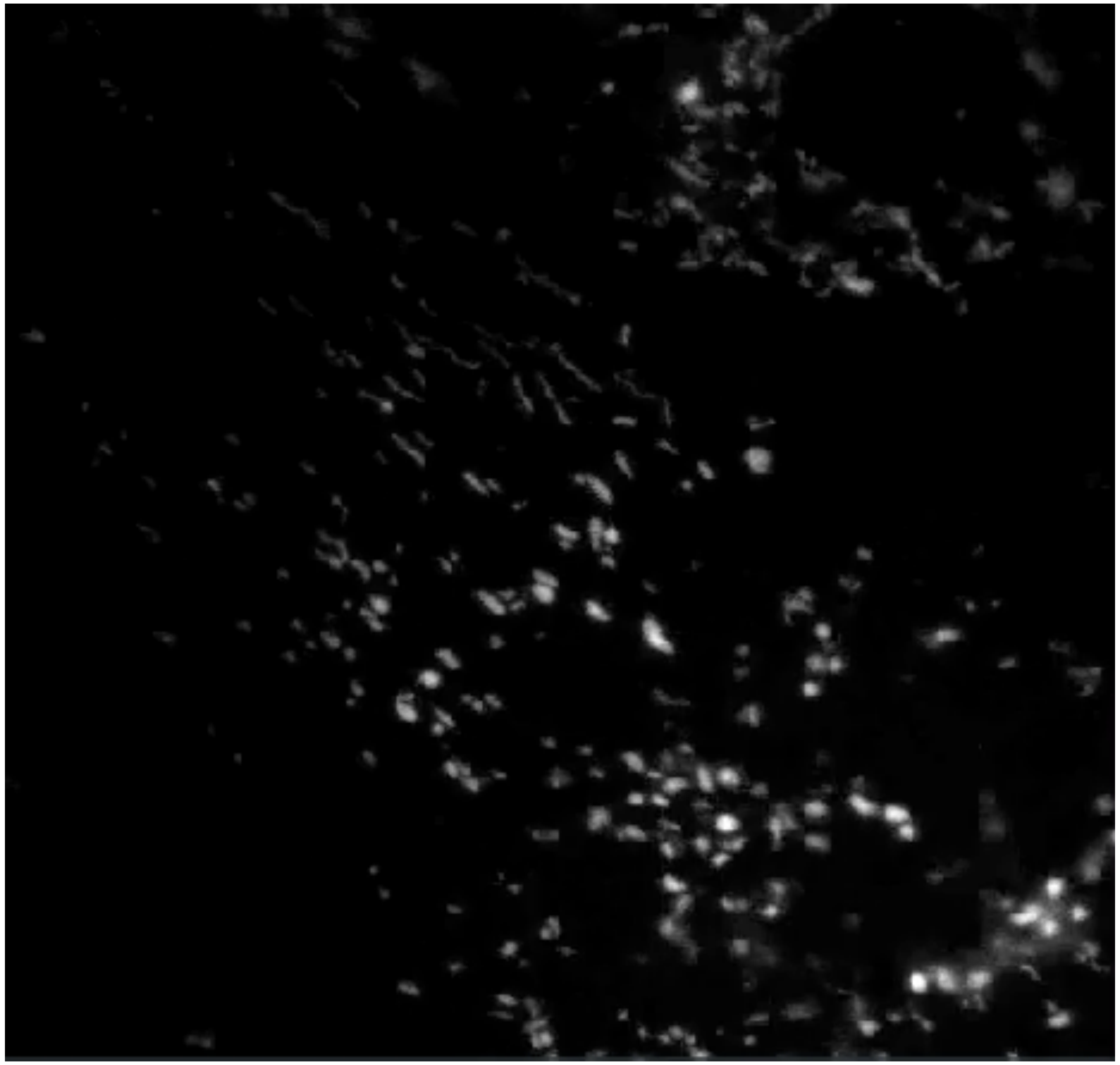
Mitochondrial dynamics: fission, fusion and mitophagy



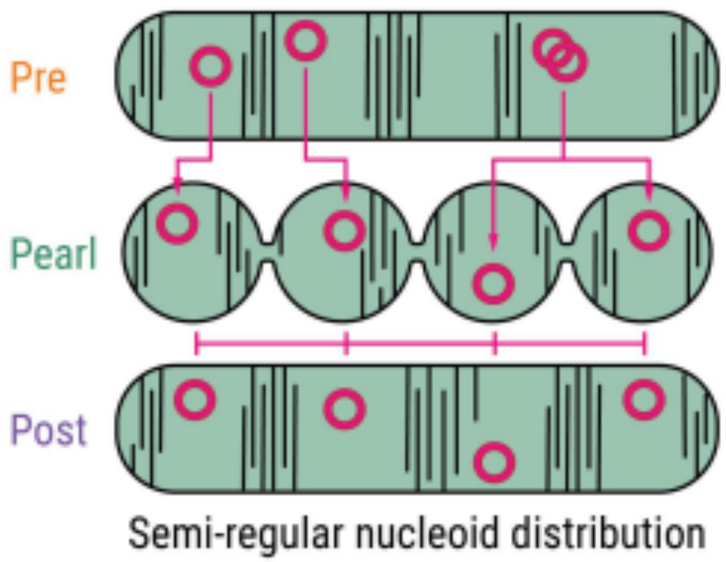


Mitochondrial dynamics: fission, fusion and mitophagy

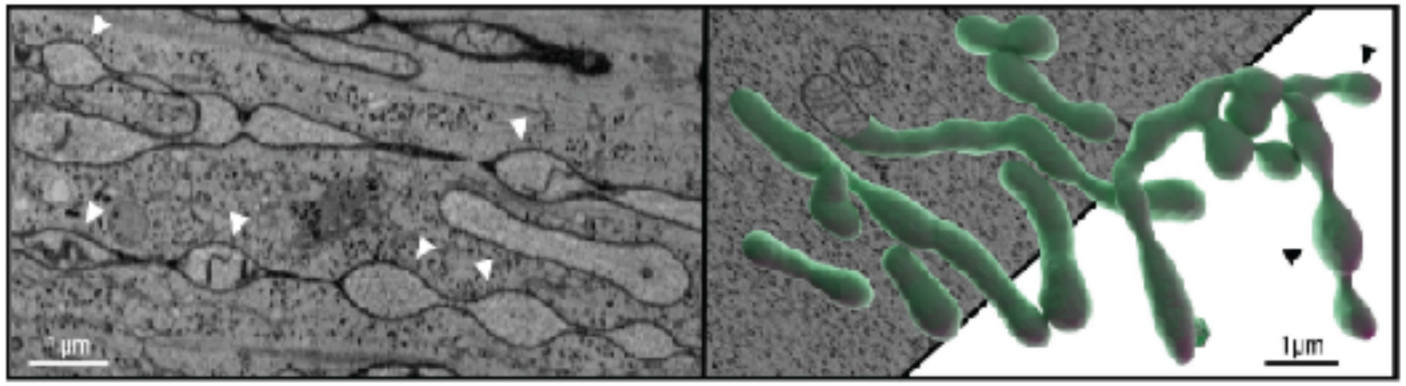




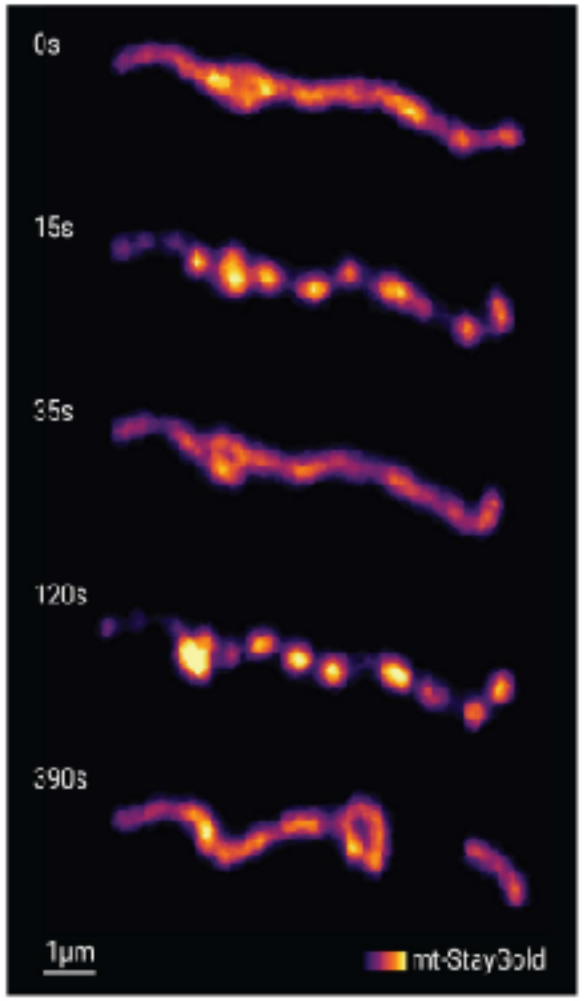
Pearling drives mitochondrial DNA nucleoid distribution



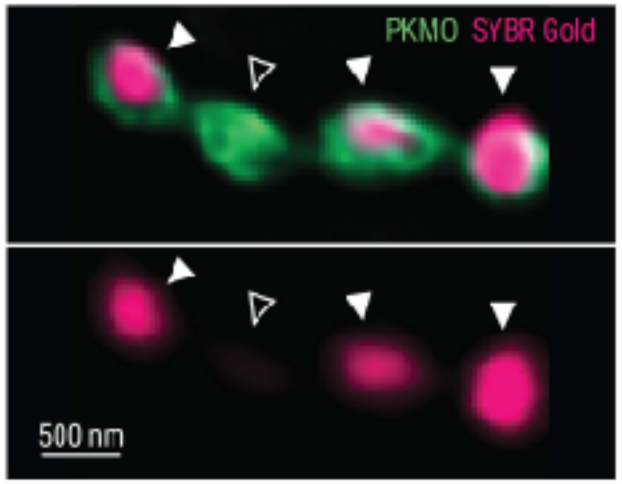
C FIB-SEM and 3D reconstruction of spontaneous mitochondrial pearling



A Spontaneous pearling

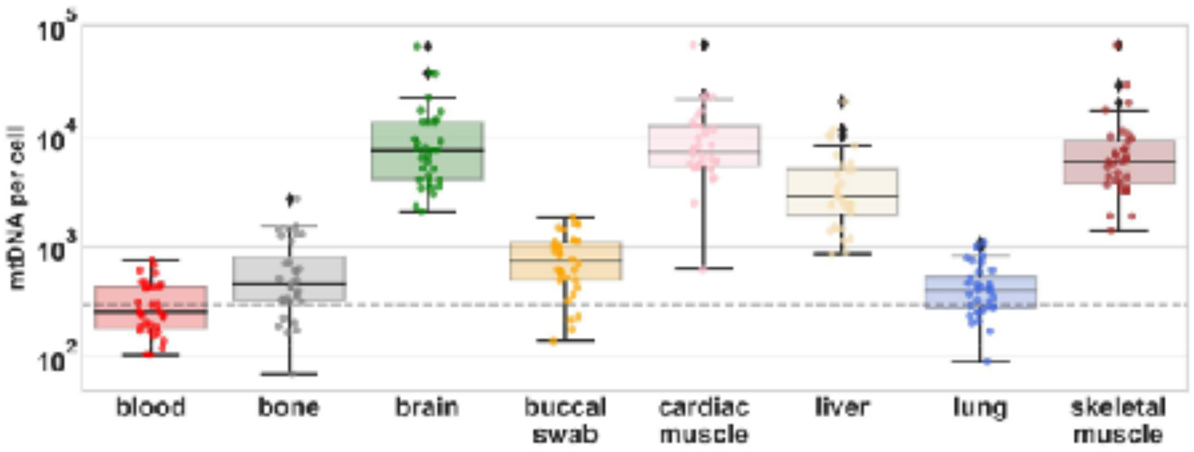


E Nucleoid-free pearls



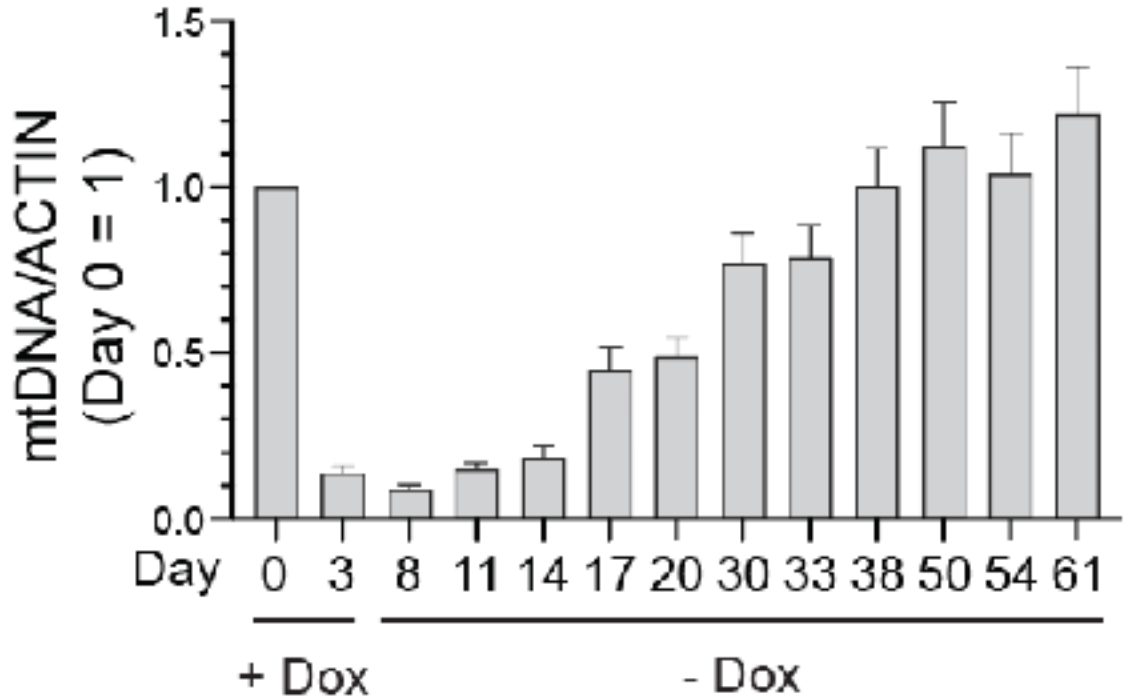
mtDNA copy number is tightly regulated

Tissue specific



Naue et al., PMID: 38040171
Chocron et al., PMID: 30419337

Homeostasis

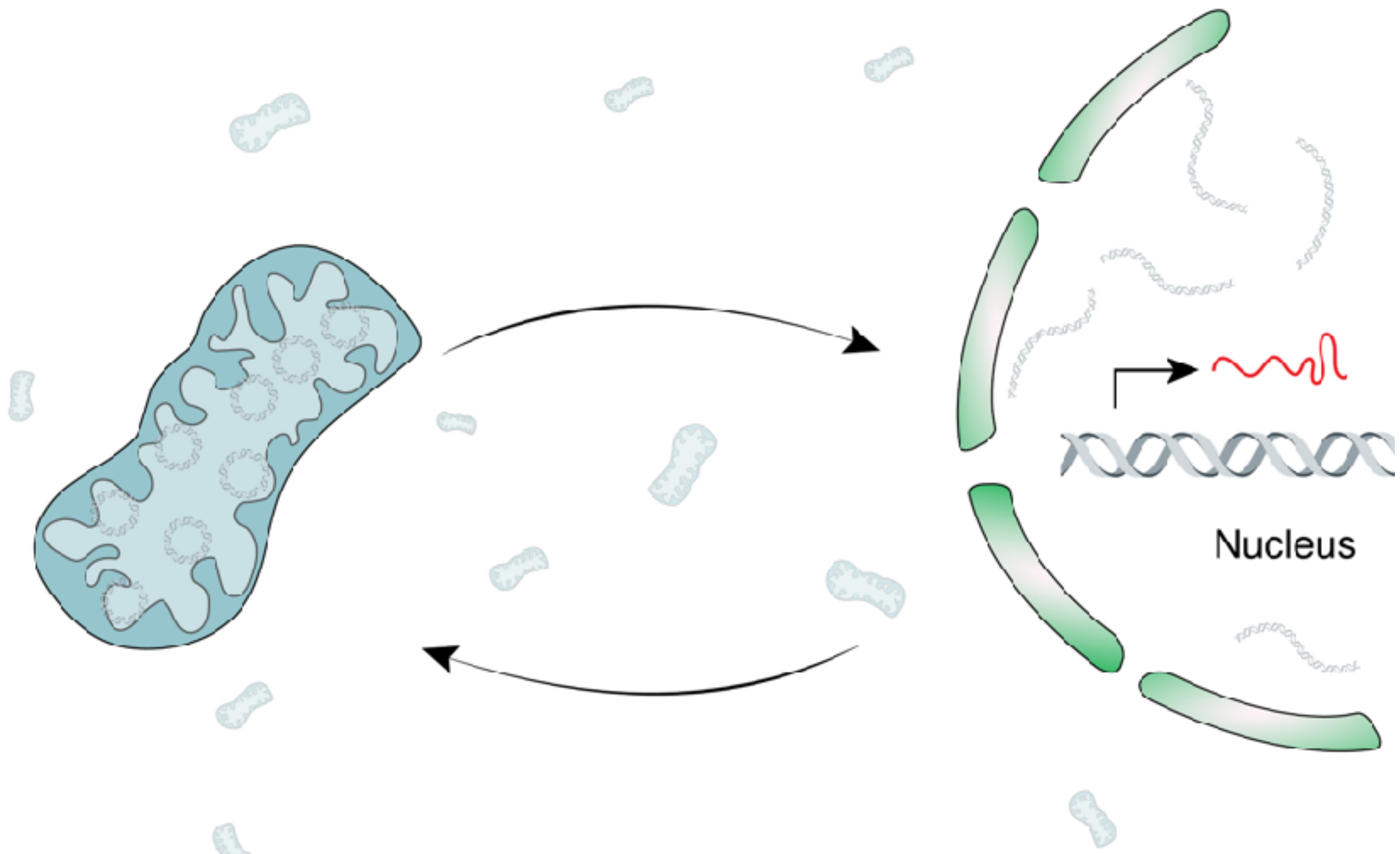


Dox inducible mito-ApaLI

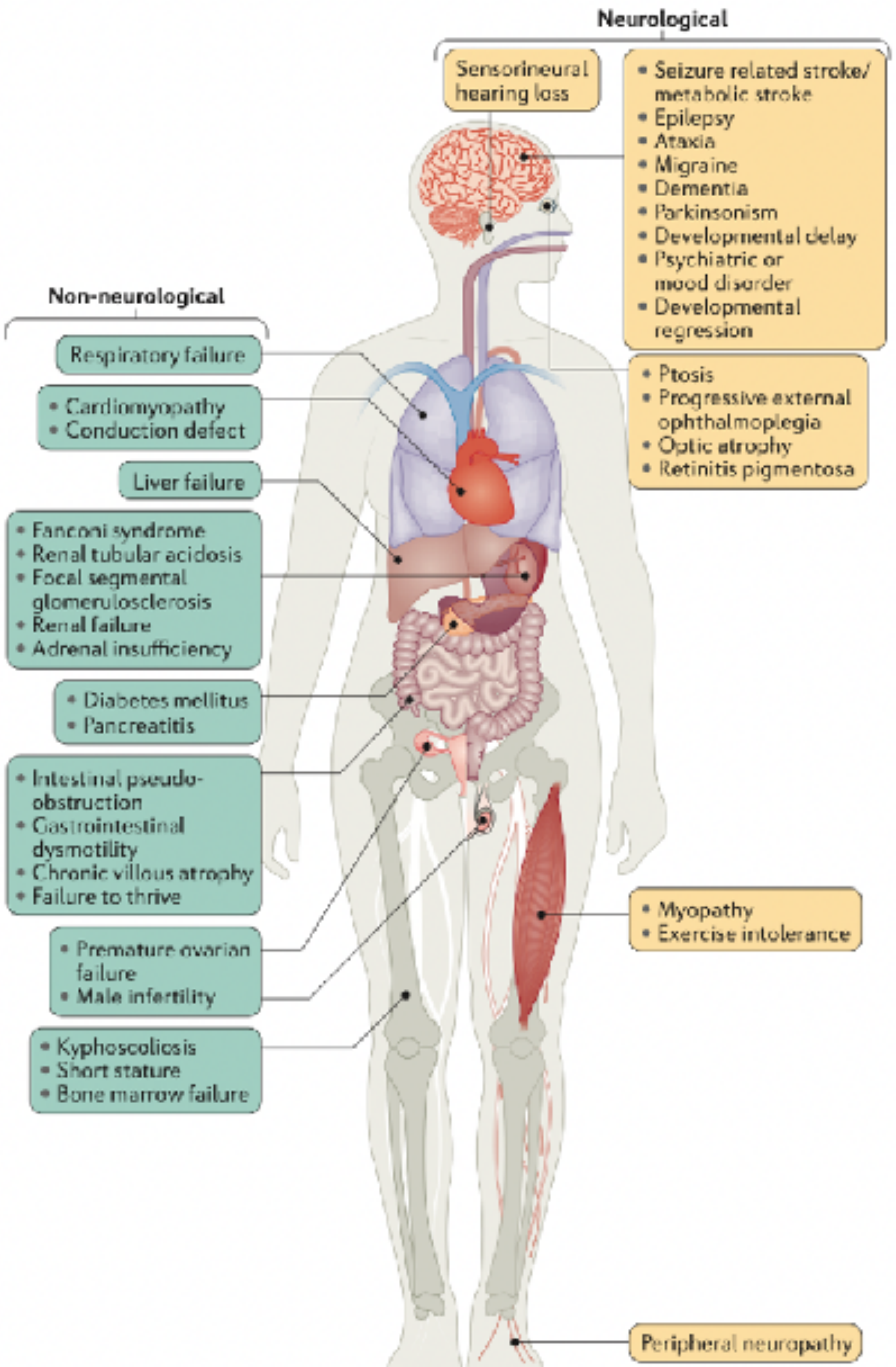
Mechanism?

Mito-nuclear crosstalk

Challenges inherent to endosymbiosis



Mitochondrial disorders



Adapted from Gorman et al., 2016

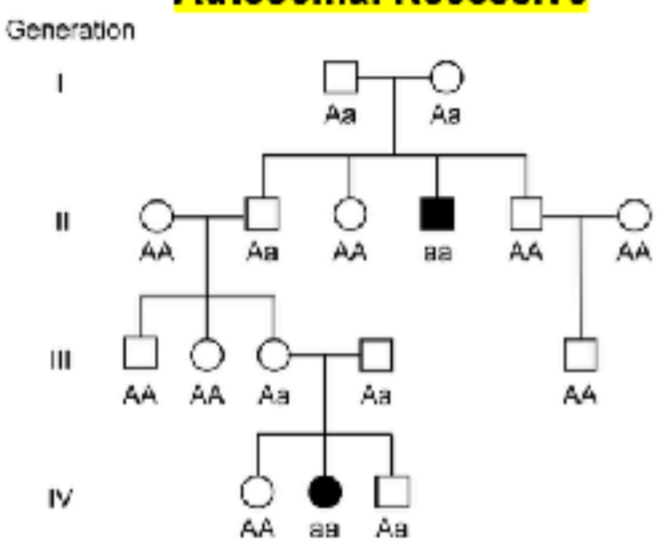
- Affect 1:4,000 newborns.
- Clinically heterogeneous, may occur at any age, and manifest with a broad range of multiple symptoms.
- Primarily tissue with high energy requirements, such as the CNS, heart tissue, and skeletal muscle.
- mtDNA mutations: point mutations, deletions, depletion
- Mutations in nuclear genes encoding mitochondrial proteins

Mitochondrial disorders: inhertience, a tale o two genomes

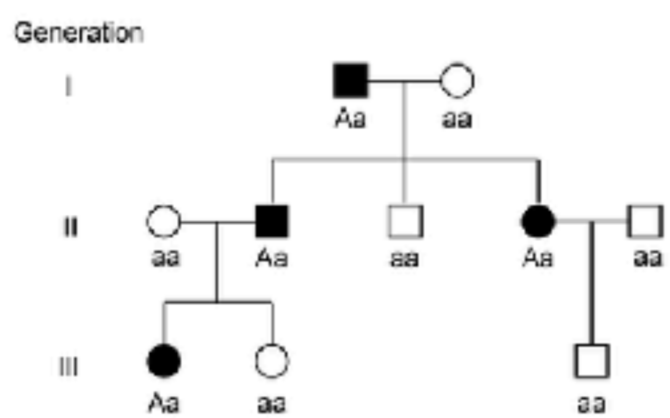
Nuclear Genome
- Mendelian Inheritance
- Dominant or Recessive

Mitochondrial Genome
- Maternal Inheritance
- *de novo* mtDNA mutations

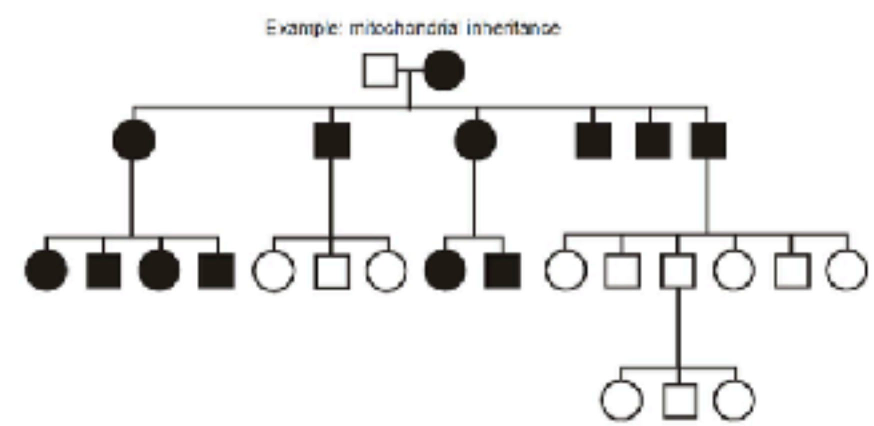
Autosomal Recessive



Autosomal Dominant



Maternal Inheritance



Classification of mitochondrial diseases

Table 1. A simplified genetic classification of mitochondrial diseases. Shown are examples of mutations (mtDNA) or disease genes (nuclear disorders).

Mitochondrial DNA mutations	
mtDNA rearrangements	Sporadic, single large-scale mtDNA deletions
Protein synthesis gene	mt-tRNA gene mutations (e.g., m.3243A>G, m.8344A>G) mt-rRNA gene mutations (e.g., m.1555A>G)
Protein-encoding gene	mt-mRNA gene mutations (e.g., m.8993T>G/C; primary LHON mutations)
Mendelian mitochondrial disorders	
OXPHOS structural gene	Mutations of complex I-V protein subunits
OXPHOS assembly factor	Mutations in proteins required for complex assembly (e.g., SURF1, BCS1L, ACAD9, TMEM70)
mtDNA maintenance gene	Mutations in proteins involved in mtDNA replication (e.g., POLG, PEO1, MGME1, RNASEH1) or dNTP synthesis/salvage (e.g., DGUOK, TK2) leading to secondary mtDNA abnormalities (multiple mtDNA deletions or mtDNA copy number loss)
mt-translation disorders	Mutations in translation and release factors (e.g., *TUFM*, *TSFM*, *C12orf65*) Mutations in mt-tRNA modifying proteins (e.g., *MTO1*, *GTPBP3*, *MTFMT*, *TRIT1*) Mutations in mRNA processing enzymes (e.g., *LRPPRC*, *ELAC2*, *PNPT1*, *MRPP2*) Mutations in mt-aminoacyl tRNA synthetases (e.g., *AARS2*, *DARS2*, *RARS2*, *EARS2*, *YARS2*, *FARS2*) Mutations in mitoribosomal proteins (e.g., *MRPS16*, *MRPS22*, *MRPL3*, *MRPL12*, *MRPL44*)
Lipid metabolism	Mutations in *AGK*, *TAZ*
Phospholipid remodeling	Mutations in *SERAC1*
Disulfide relay system	Mutations in *GFER*
Mitochondrial homeostasis	Mutations in *FBXL4*, *CLPB*
Fe-S cluster assembly/homeostasis	Mutations in *ISCU*, *BOLA3*, *NFU1*, *IBA57*, *LYRM4*
Mitochondrial fission/fusion	Mutations in *OPA1*, *MFN2*, *DLP1*
Mitochondrial protein import	Mutations in *TIMM8A*, *DNAJC19*
Apoptosis	Mutations in *AIF1*, *APOPT1*, *FASTKD2*
Coenzyme Q10 biogenesis	Mutations in *COQ2*, *COQ4*, *COQ9*, *PDSS1*, *PDSS2*, *CABC1*, *ADCK3*
Mitochondrial chaperones	Mutations in *SPG7*, *AFGL32*
Mitochondrial metabolism	Mutations in *ETHE1*, *HIBCH*, *ECHS1*

lightowlers et al, 2015

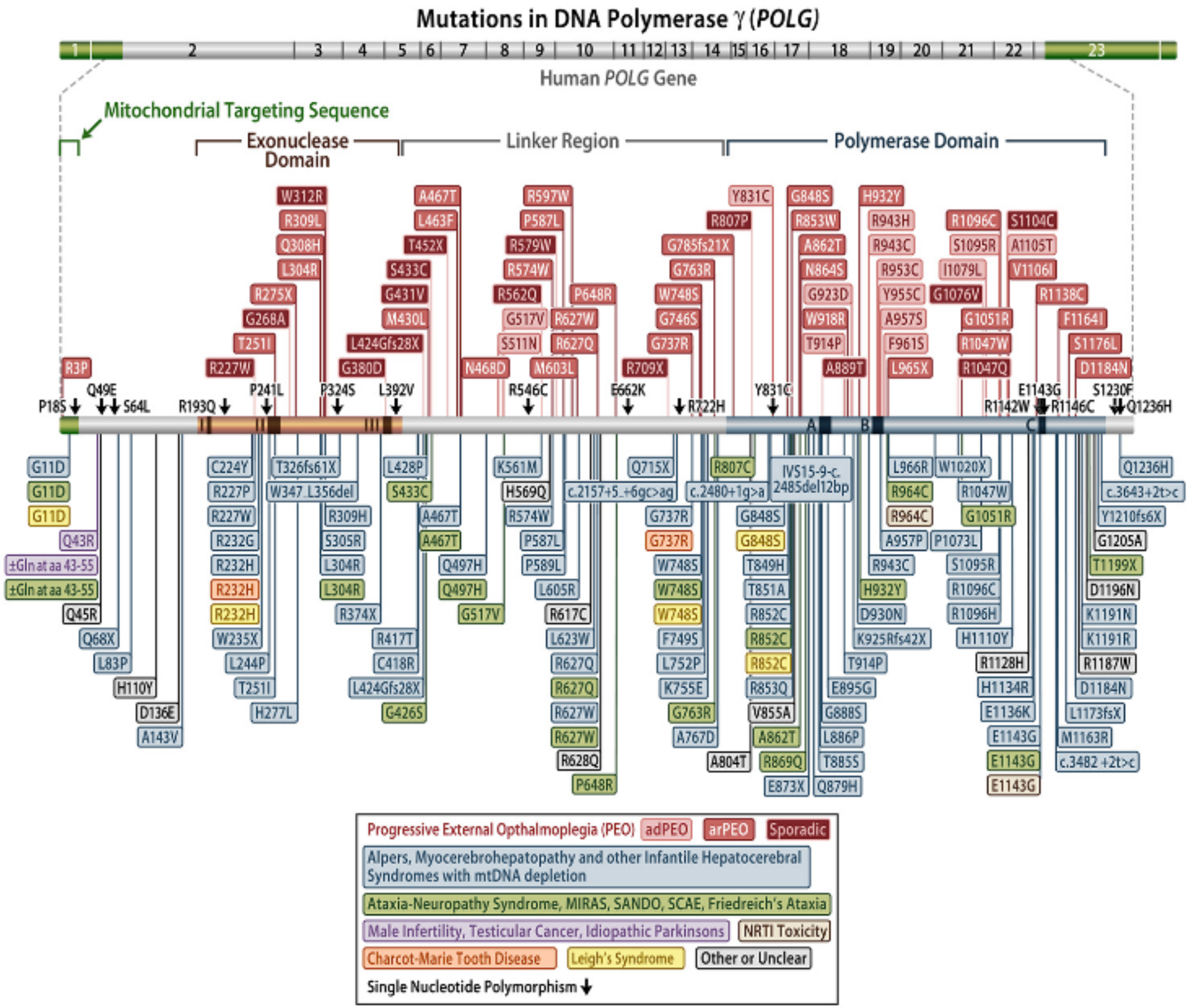
Overview of Clinical disorders

Table 2 | **Clinical disorders that are caused by mutations in mitochondrial DNA**

Mitochondrial DNA disorder	Clinical phenotype	mtDNA genotype	Gene	Status	Inheritance	Reference
Kearns–Sayre syndrome	Progressive myopathy, ophthalmoplegia, cardiomyopathy	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	61,158
CPEO	Ophthalmoplegia	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	61, 64
Pearson syndrome	Pancytopoenia, lactic acidosis	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	65
MELAS	Myopathy, encephalopathy lactic acidosis, stroke-like episodes	3243A>G; 3271T>C	*TRNL1*	Heteroplasmic	Maternal	159
		Individual mutations	*ND1 and ND5*	Heteroplasmic	Maternal	160, 161
MERRF	Myoclonic epilepsy, myopathy	8344A>G; 8356T>C	*TRNK*	Heteroplasmic	Maternal	162
NARP	Neuropathy, ataxia, retinitis pigmentosa	8993T>G	*ATP6*	Heteroplasmic	Maternal	163
MILS	Progressive brain-stem disorder	8993T>C	*ATP6*	Heteroplasmic	Maternal	67
MIDD	Diabetes, deafness	3243A>G	*TRNL1*	Heteroplasmic	Maternal	164
LHON	Optic neuropathy	3460G>A 11778G>A 14484T>C	*ND1* *ND4* *ND6*	Hetero- or homoplasmic Hetero- or homoplasmic Hetero- or homoplasmic	Maternal Maternal Maternal	165 62 166
Myopathy and diabetes	Myopathy, weakness, diabetes	14709T>C	*TRNE*	Hetero- or homoplasmic	Maternal	167,168
Sensorineural hearing loss	Deafness	1555A>G Individual mutations	*RNR1* *TRNS1*	Homoplasmic Hetero- or homoplasmic	Maternal Maternal	55 169,170
Exercise intolerance	Fatigue, muscle weakness	Individual mutations	*CYB*	Heteroplasmic	Sporadic	68
Fatal, infantile encephalopathy; Leigh/Leigh-like syndrome	Encephalopathy, lactic acidosis	10158T>C; 10191T>C	*ND3*	Heteroplasmic	Sporadic	66

Taylor and Turnball, 2005

POLG: most frequently mutated gene



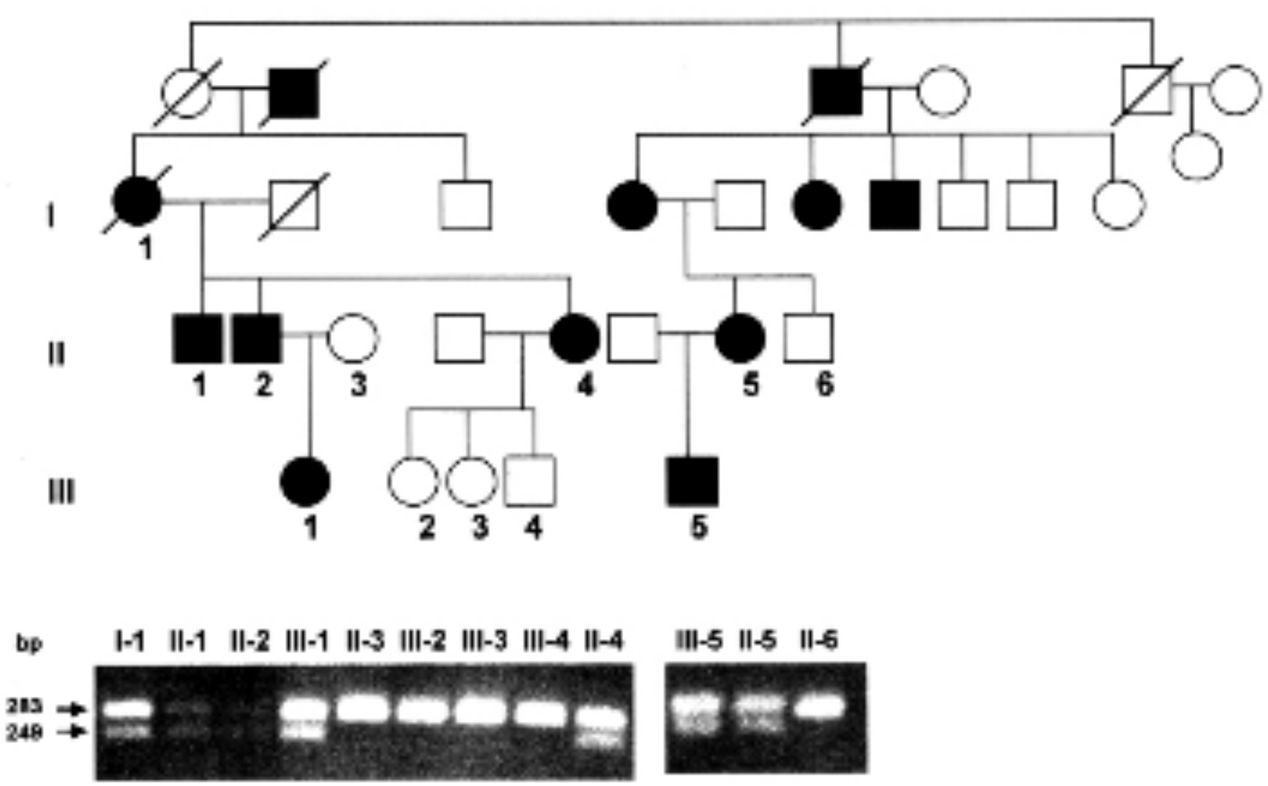
http://tools.niehs.nih.gov/polg/_assets/documents/POLG-mutation-large.pdf

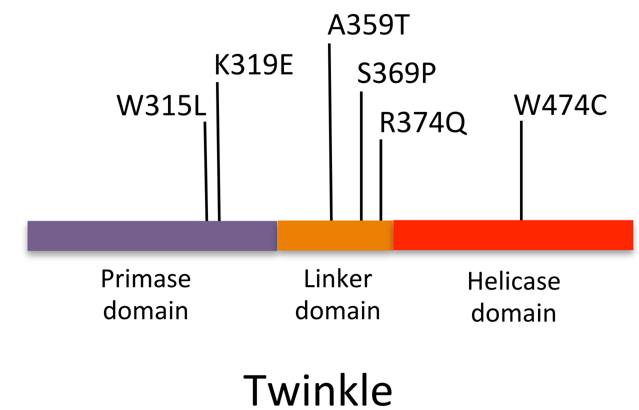
Twinkle mutations

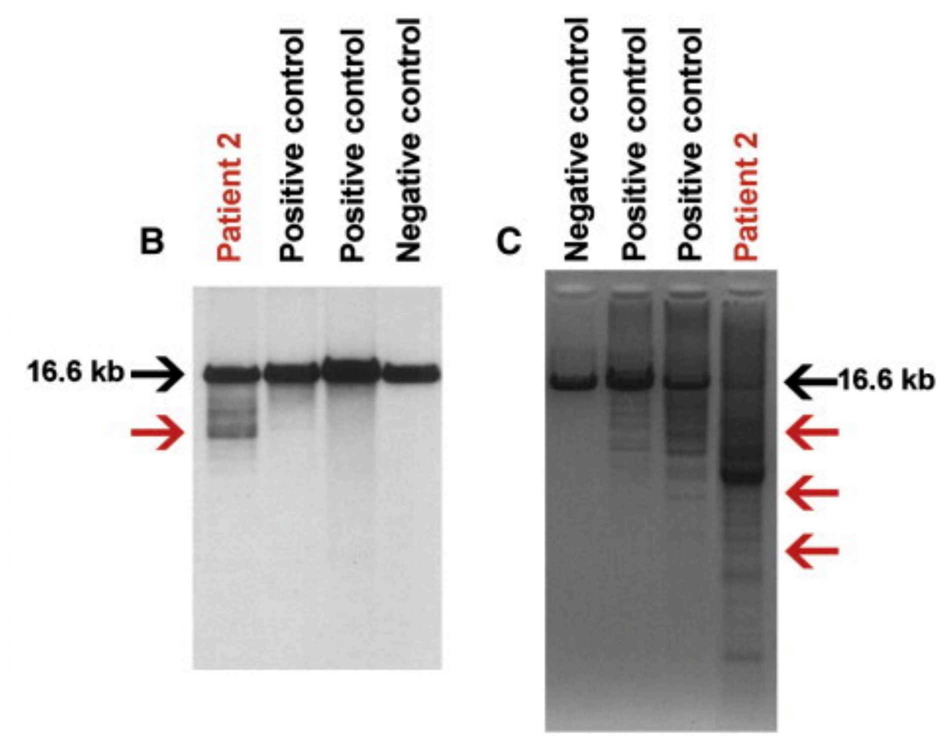
Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria

 article

Johannes N. Spelbrink[1*],



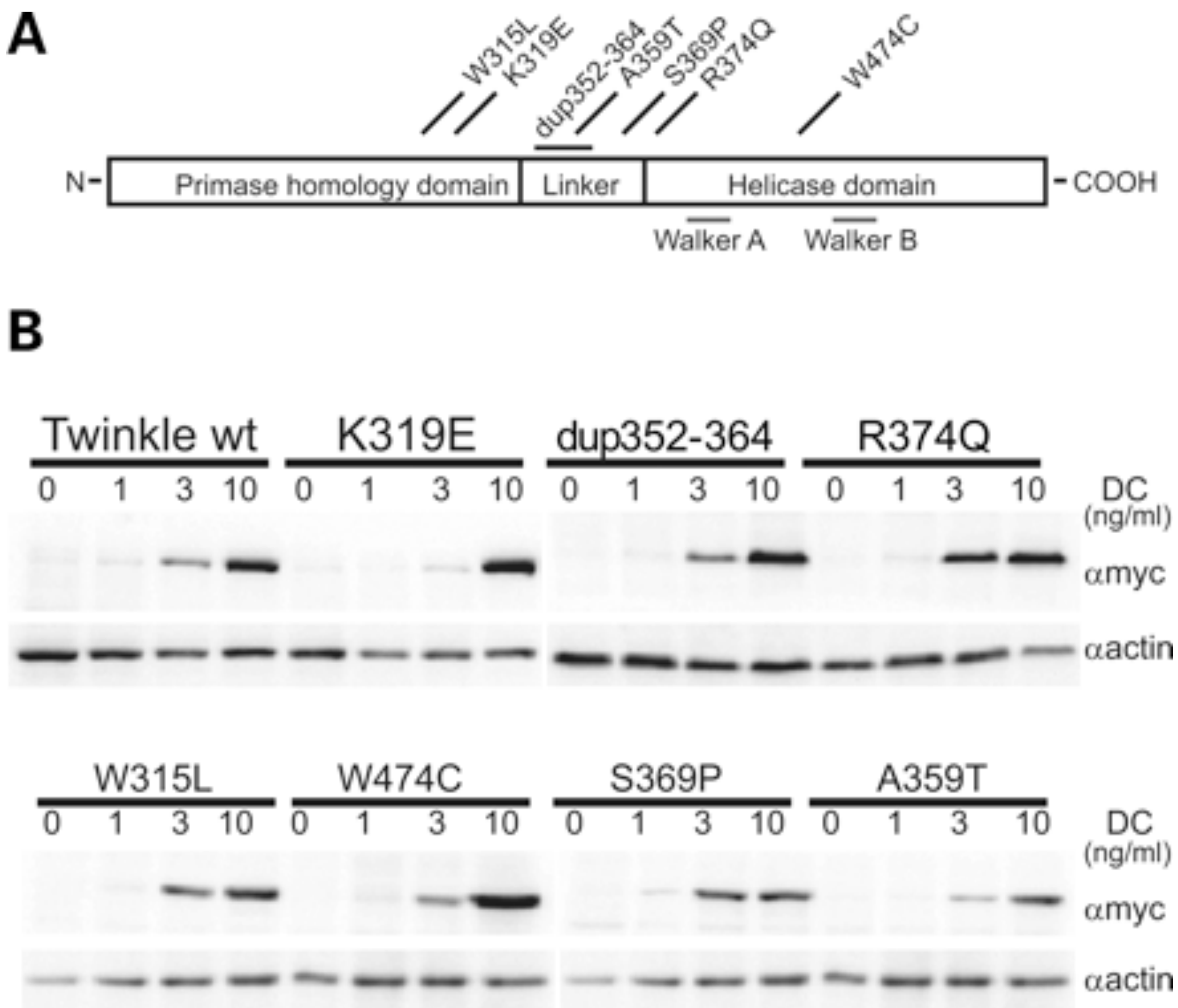


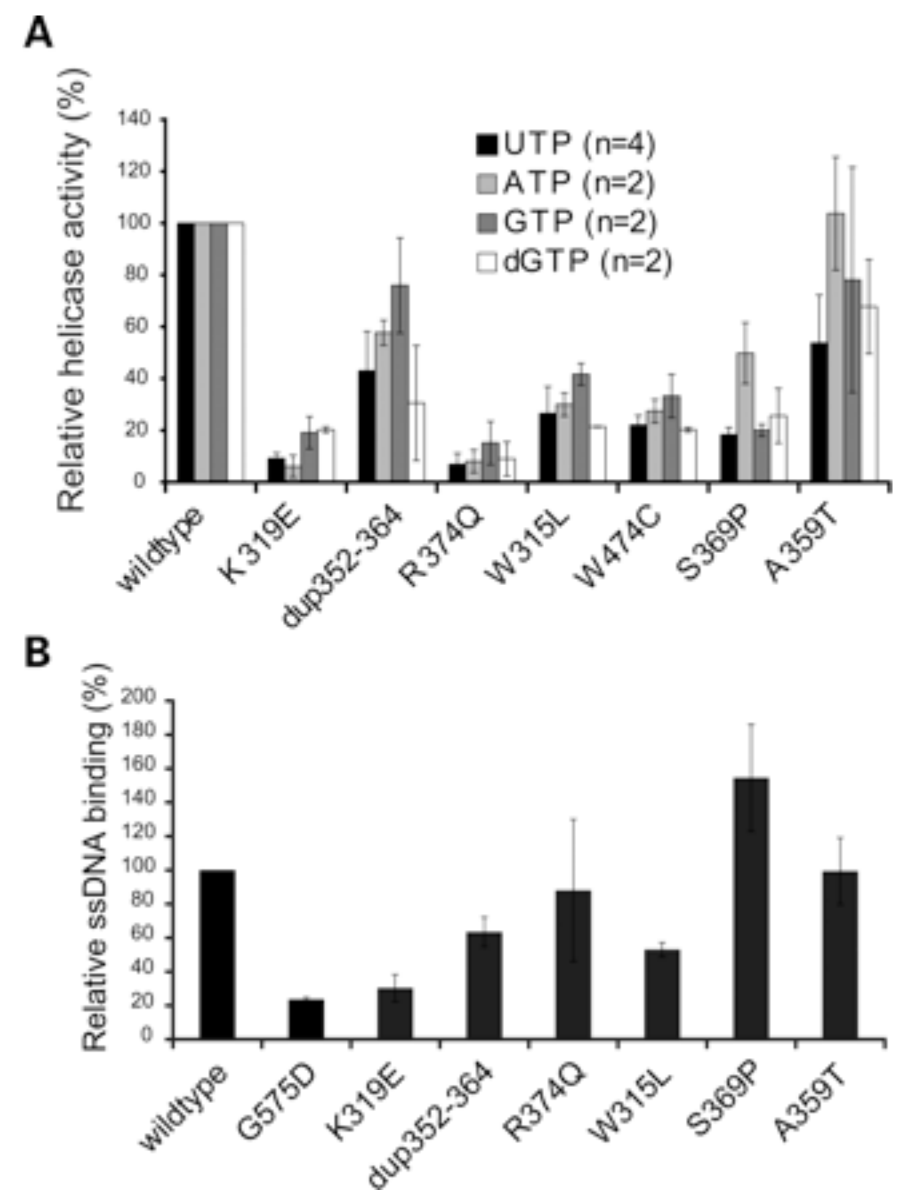


J Clin Inv. (1989) 86, 1601-1609
J Neur Sci. (2011) 308, 173-176

Twinkle mutations

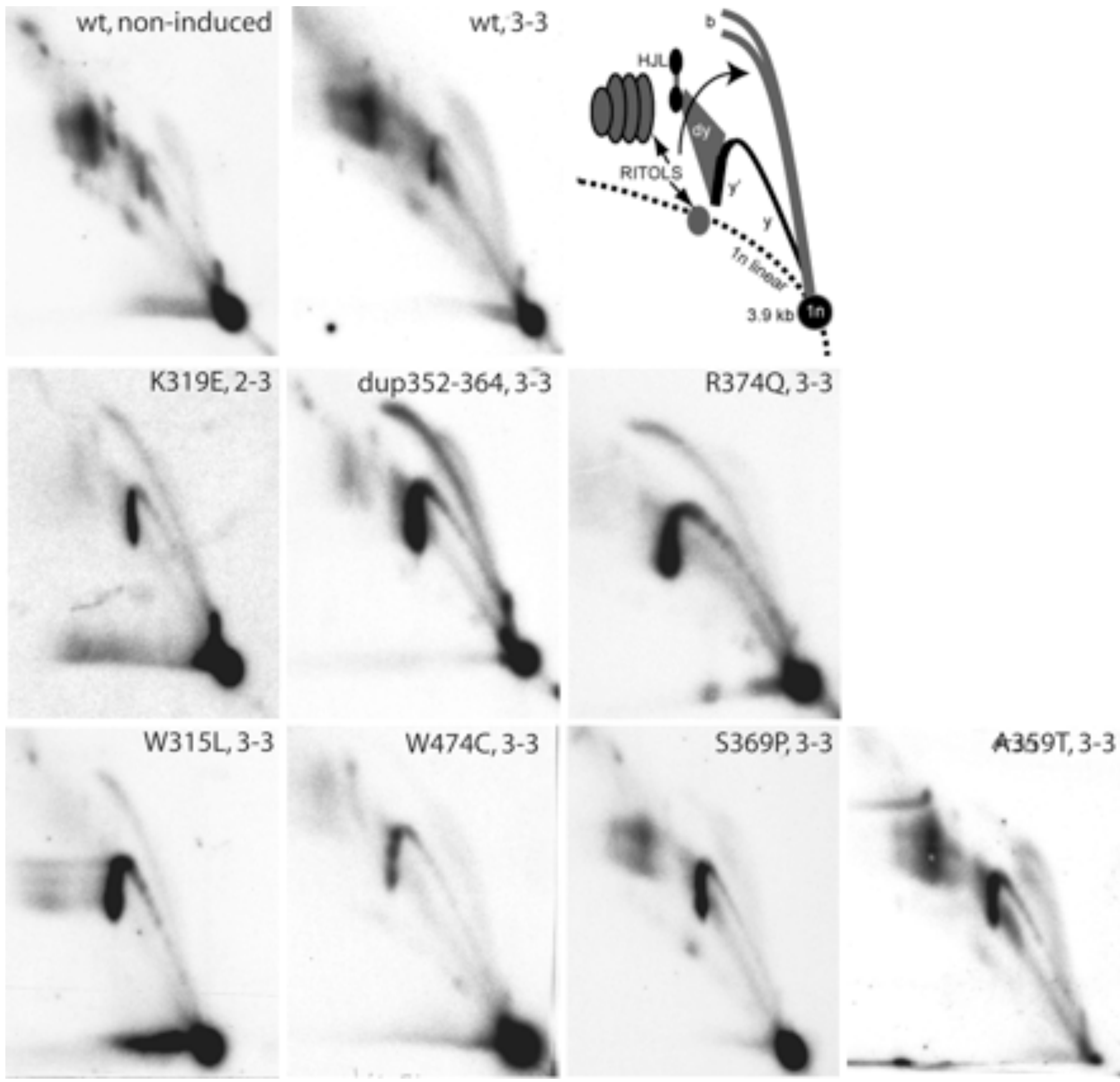
Impaired helicase activity



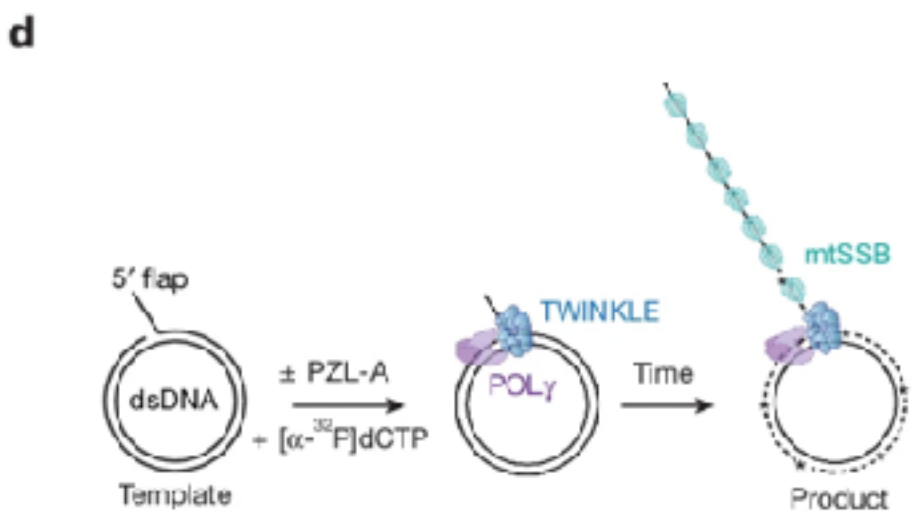


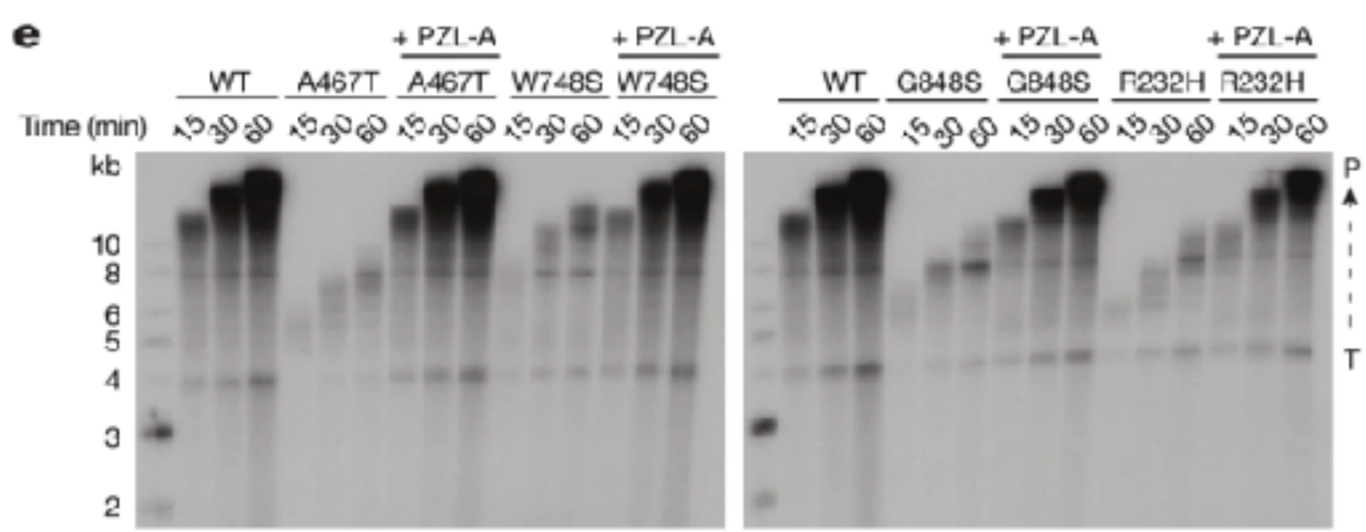
Twinkle mutations

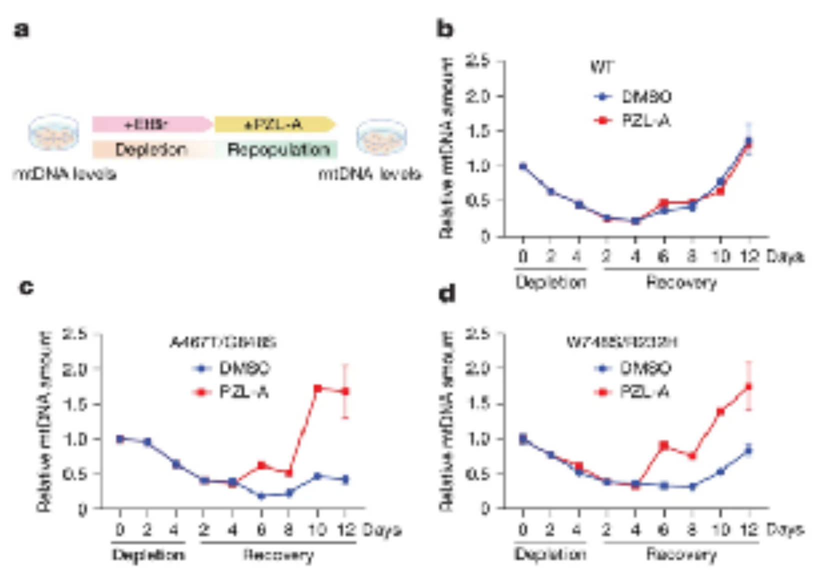
MtDNA replication is stalling upon Twinkle mutant expression.

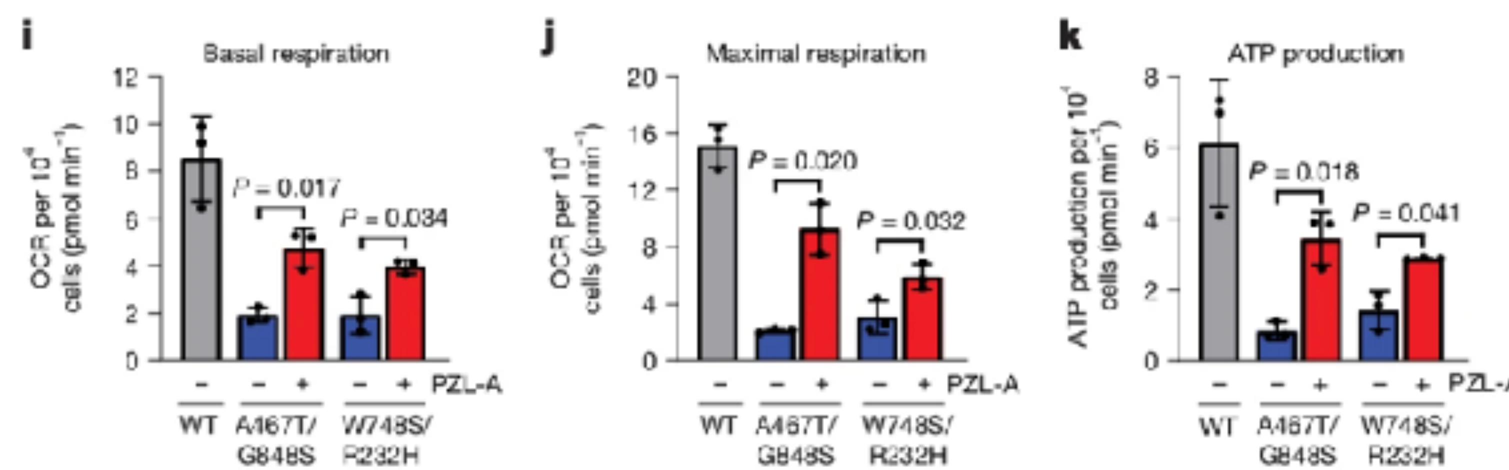


Small molecules restore mutant mitochondrial DNA polymerase activity



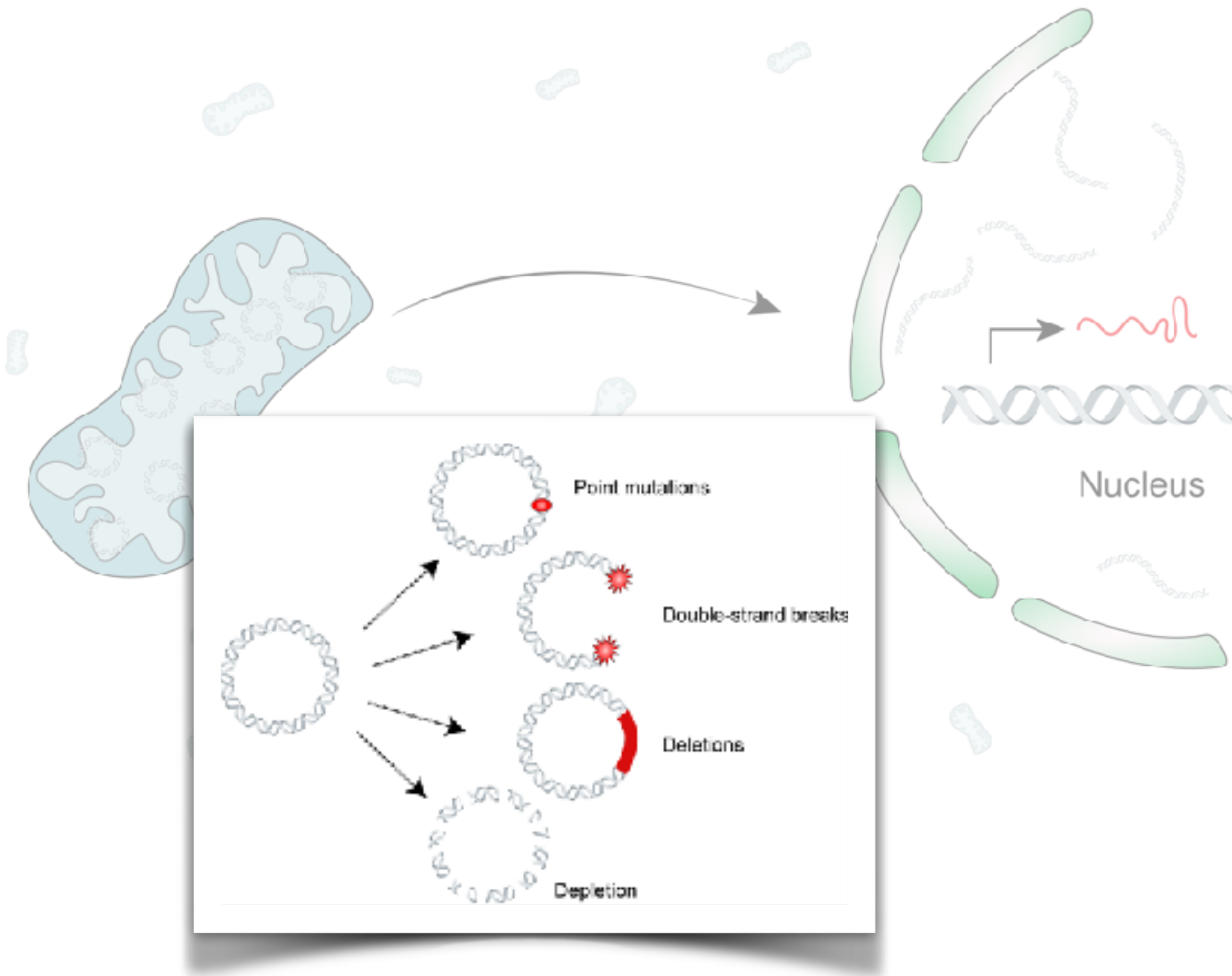




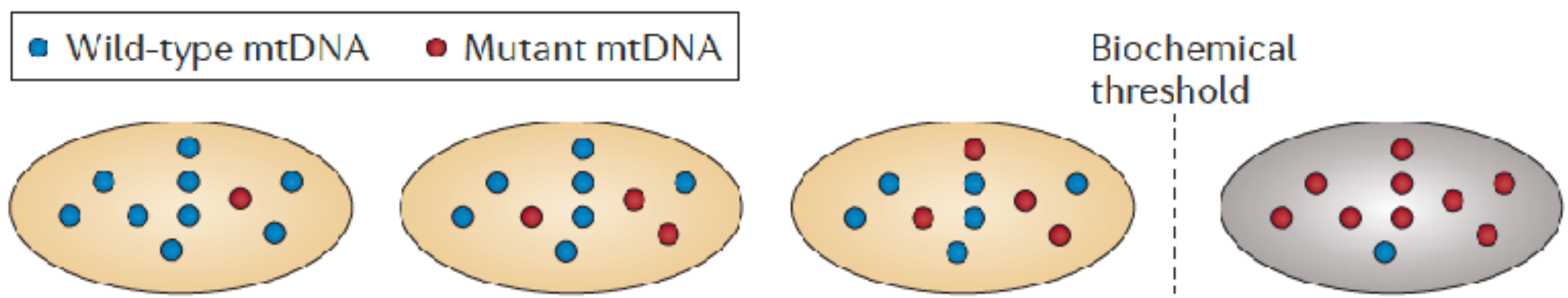


Sebastian Valenzuela et al., Nature 2025 PMID: 40205042

Types of mtDNA aberrations



mtDNA hetroplasmy and the threshold effect



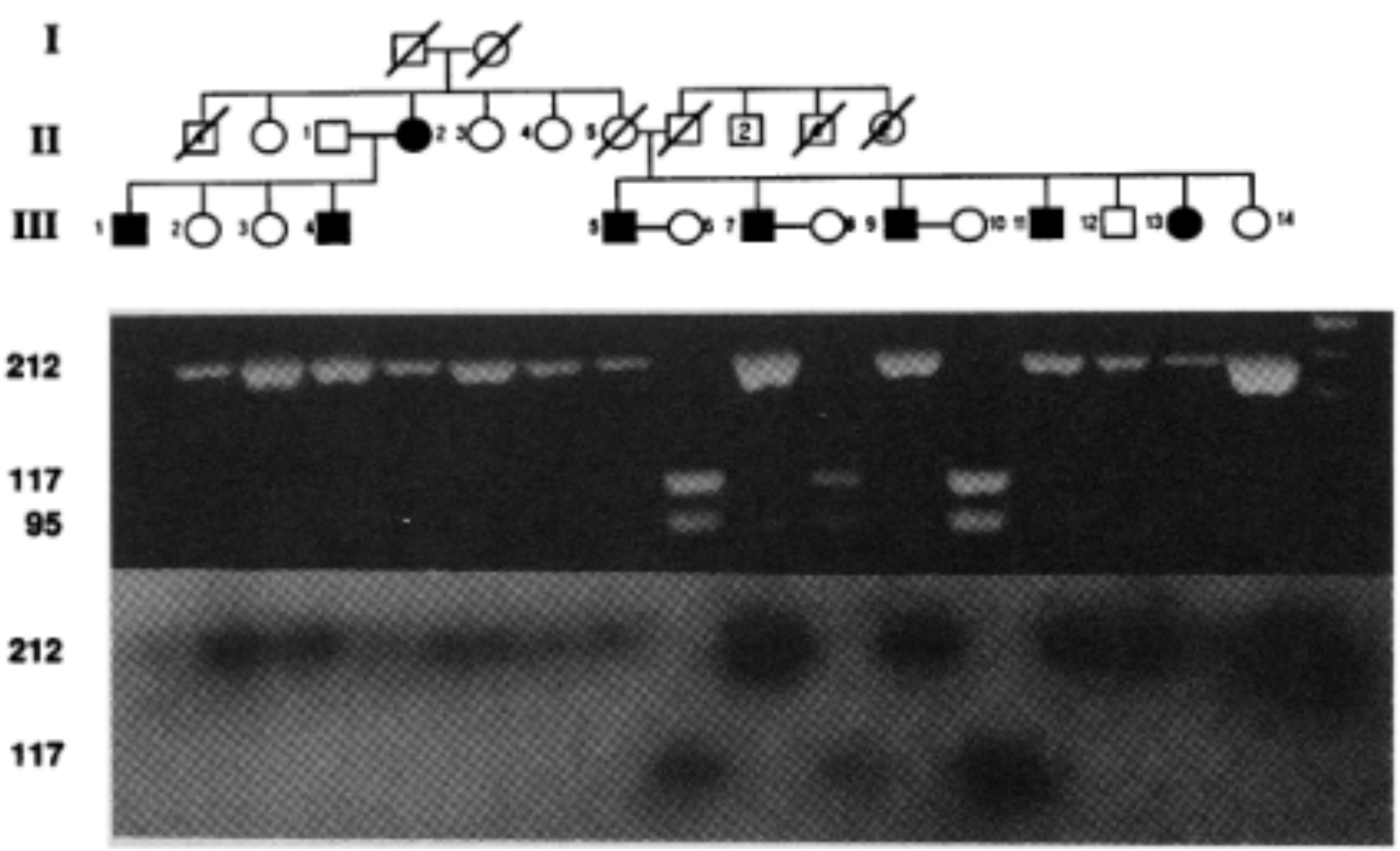
Stewart and Chinnery, 2015

First mtDNA point mutation to be identified

Mitochondrial DNA Mutation Associated with Leber's Hereditary Optic Neuropathy

9 DECEMBER 1988

DOUGLAS C. WALLACE,* GURPARKASH SINGH, MARIE T. LOTT, JUDY A. HODGE, THEODORE G. SCHURR, ANGELA M. S. LEZZA, LOUIS J. ELSAS II, EEVA K. NIKOSKELAINEN



Leber hereditary optic neuropathy (LHON) is an inherited form of vision loss.

(m.11778A>G). homoplasmic point mutation in MTND4

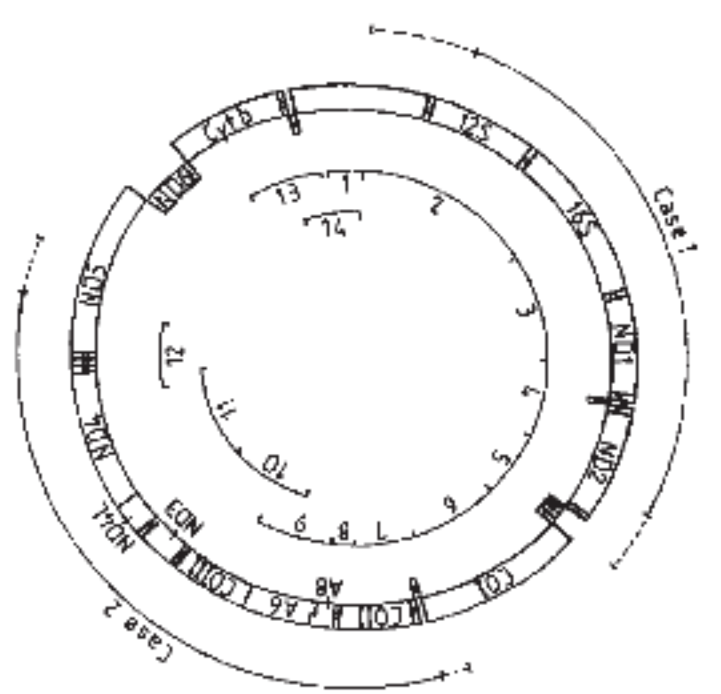
First mtDNA deletion to be identified

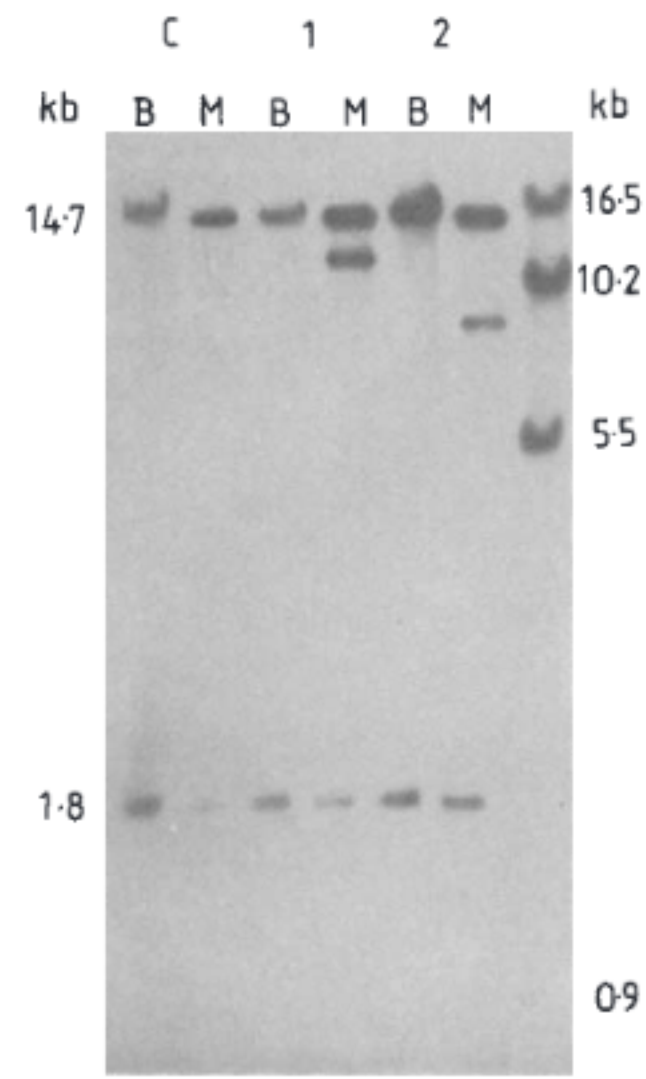
Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies

I. J. Holt, A. E. Harding & J. A. Morgan-Hughes

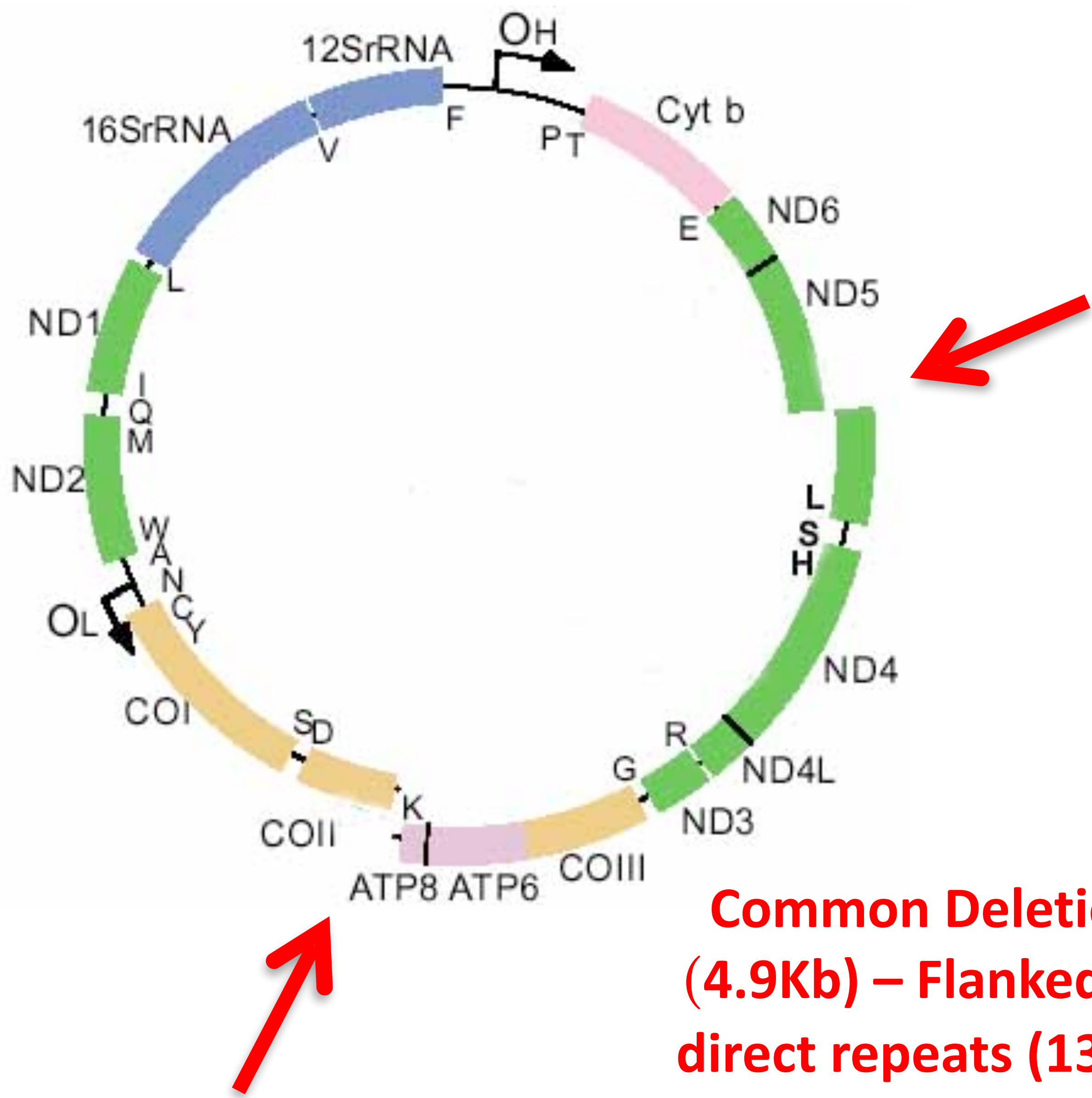
Department of Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, UK

NATURE VOL. 331 25 FEBRUARY 1988





the “common deletion”



Common Deletion (4.9Kb) – Flanked by direct repeats (13bp)

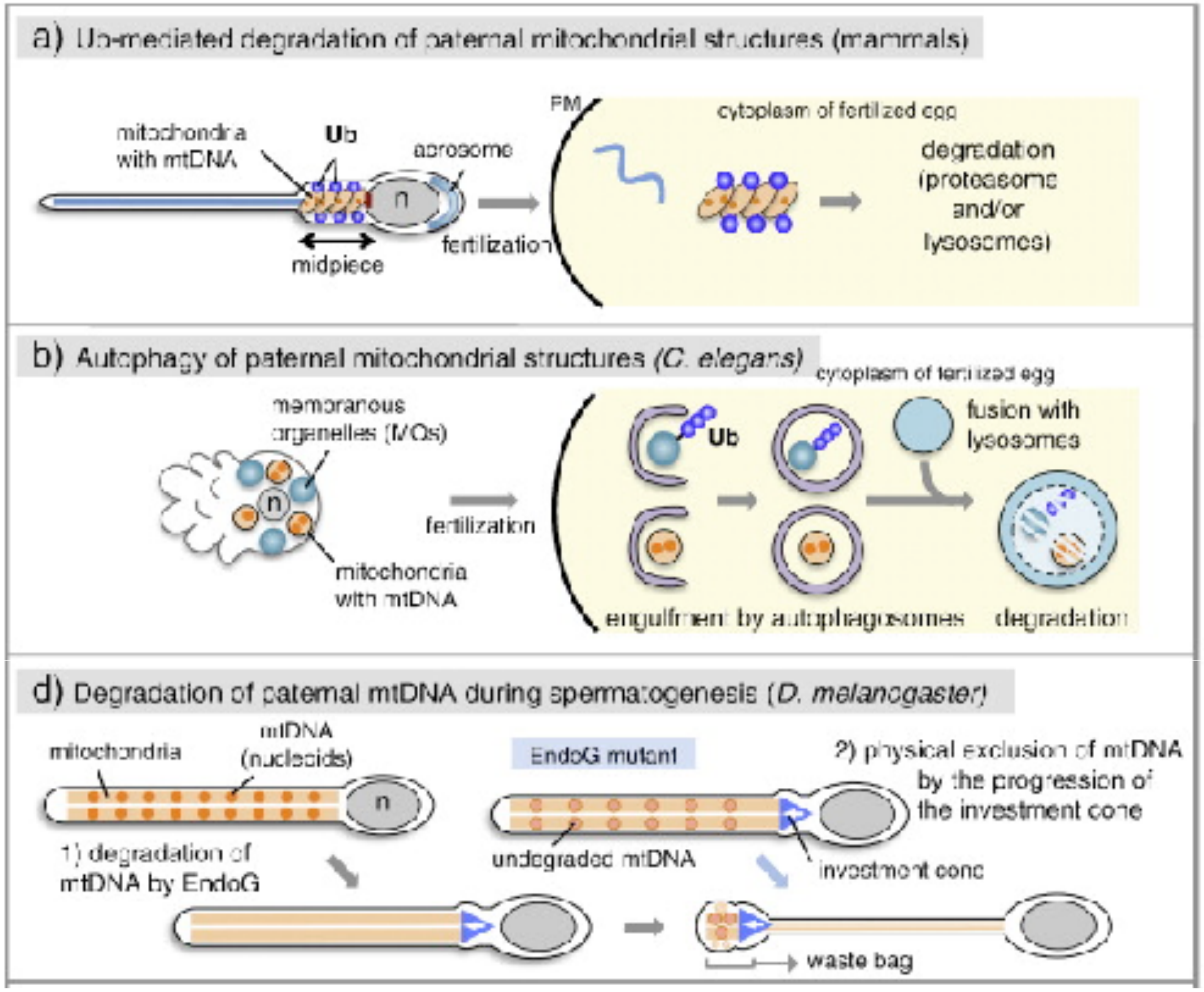
- Kearns-Sayre syndrome
- Pearson marrow syndrome
- Progressive external ophthalmoplegia

- Premature aging
-- Cardiomyopathy
- Parkinson’s disease

- The mutation rate in mammalian mitochondria is approximately 10^{-4}.
- Approximately 1 in 200 people harbor a deleterious variant of mitochondrial DNA.
- 25-65% of individuals with mtDNA variants reach moderate heteroplasmy.
- MtDNA is uniparental and does not recombine.

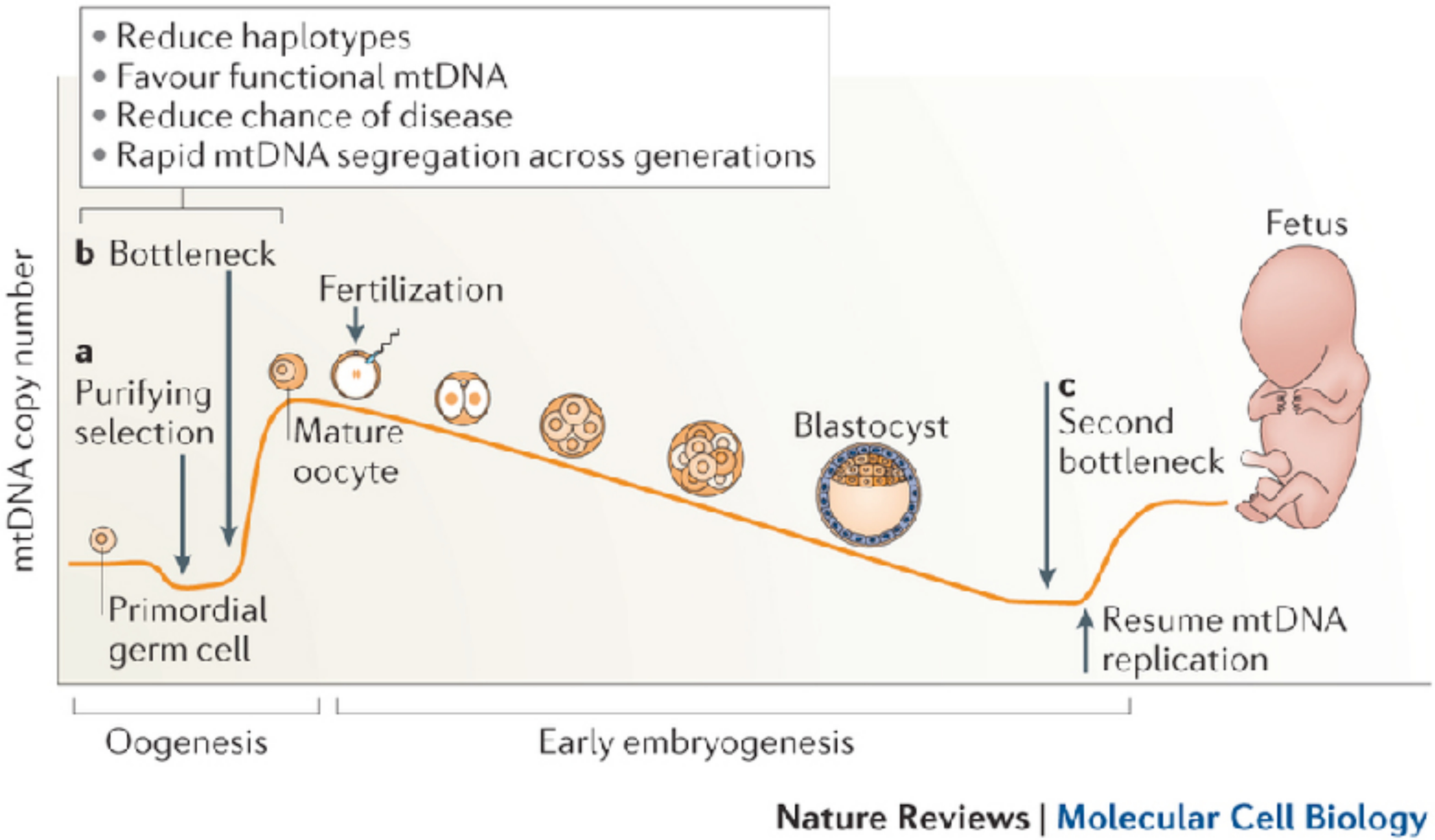
Why don’t we have more disease manifestations?

Mechanisms ensuring the elimination of paternal mtDNA



Sato et al., 2013

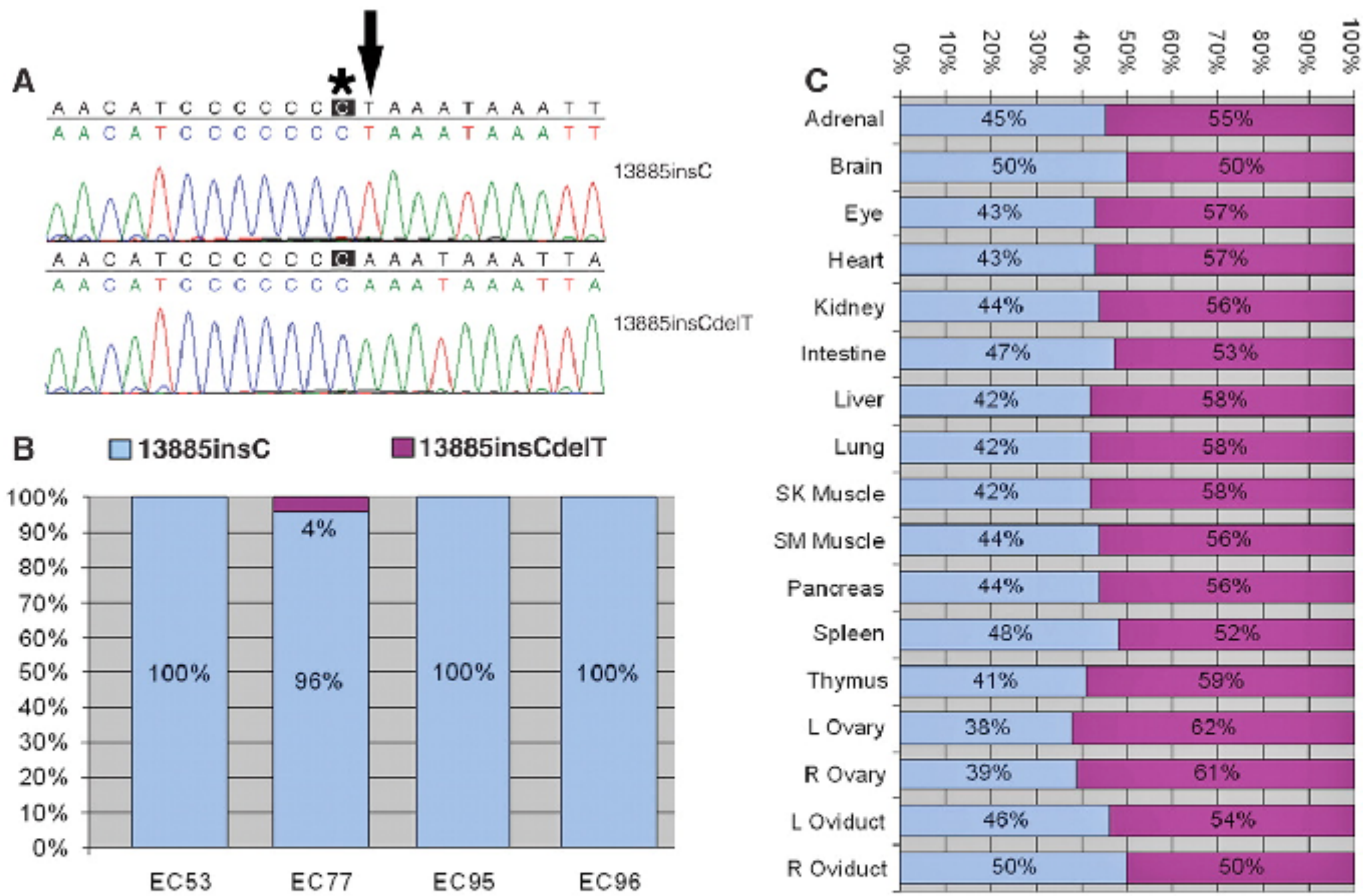
mtDNA segregation during maternal transmission and early embryogenesis



Bottleneck & selection

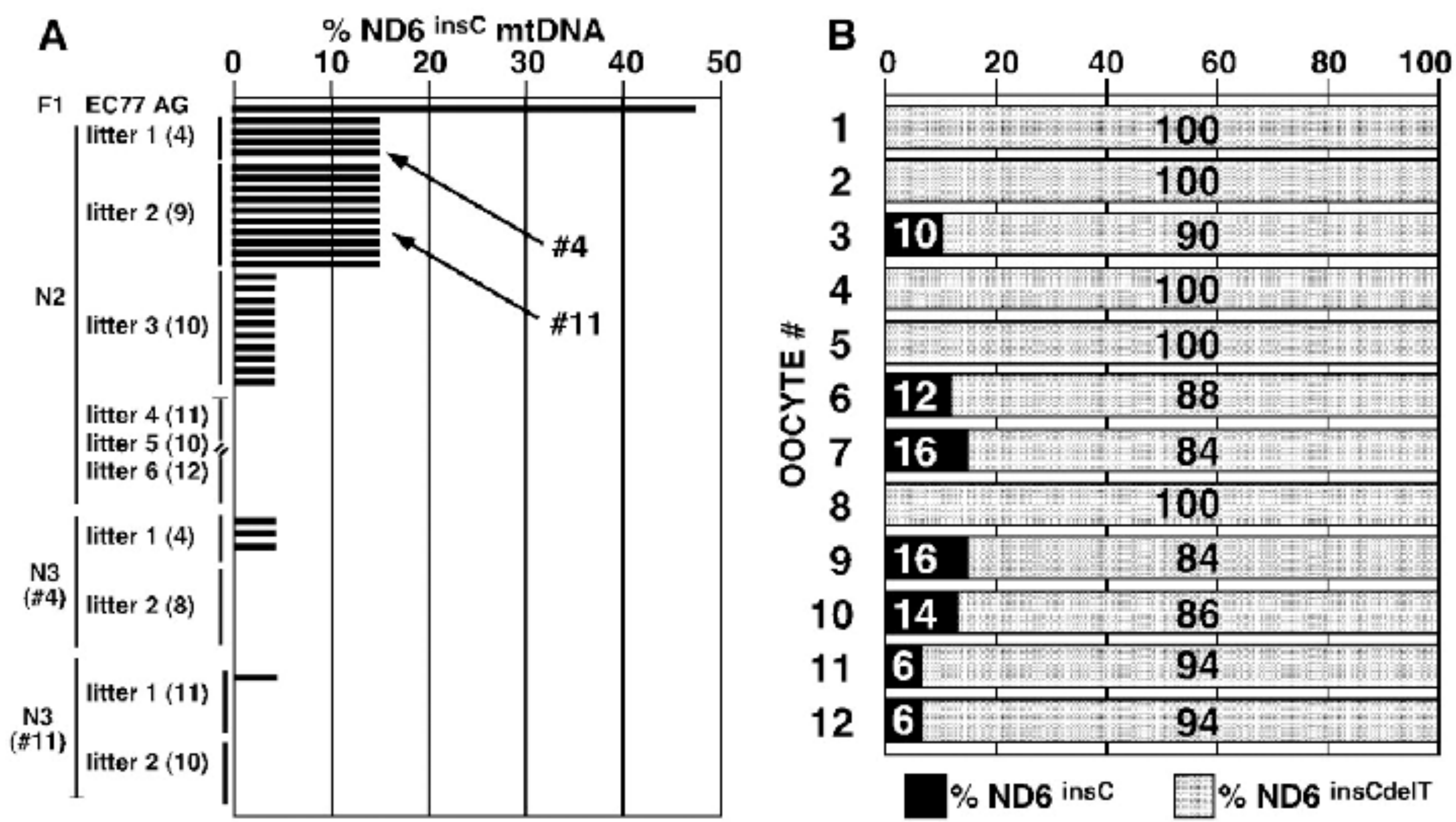
Mishra et al., 2014

A Mouse Model of Mitochondrial Disease Reveals Germline Selection Against Severe mtDNA Mutations



Fan …wallace, 2008

Selective elimination of ND6 frameshift mtDNA

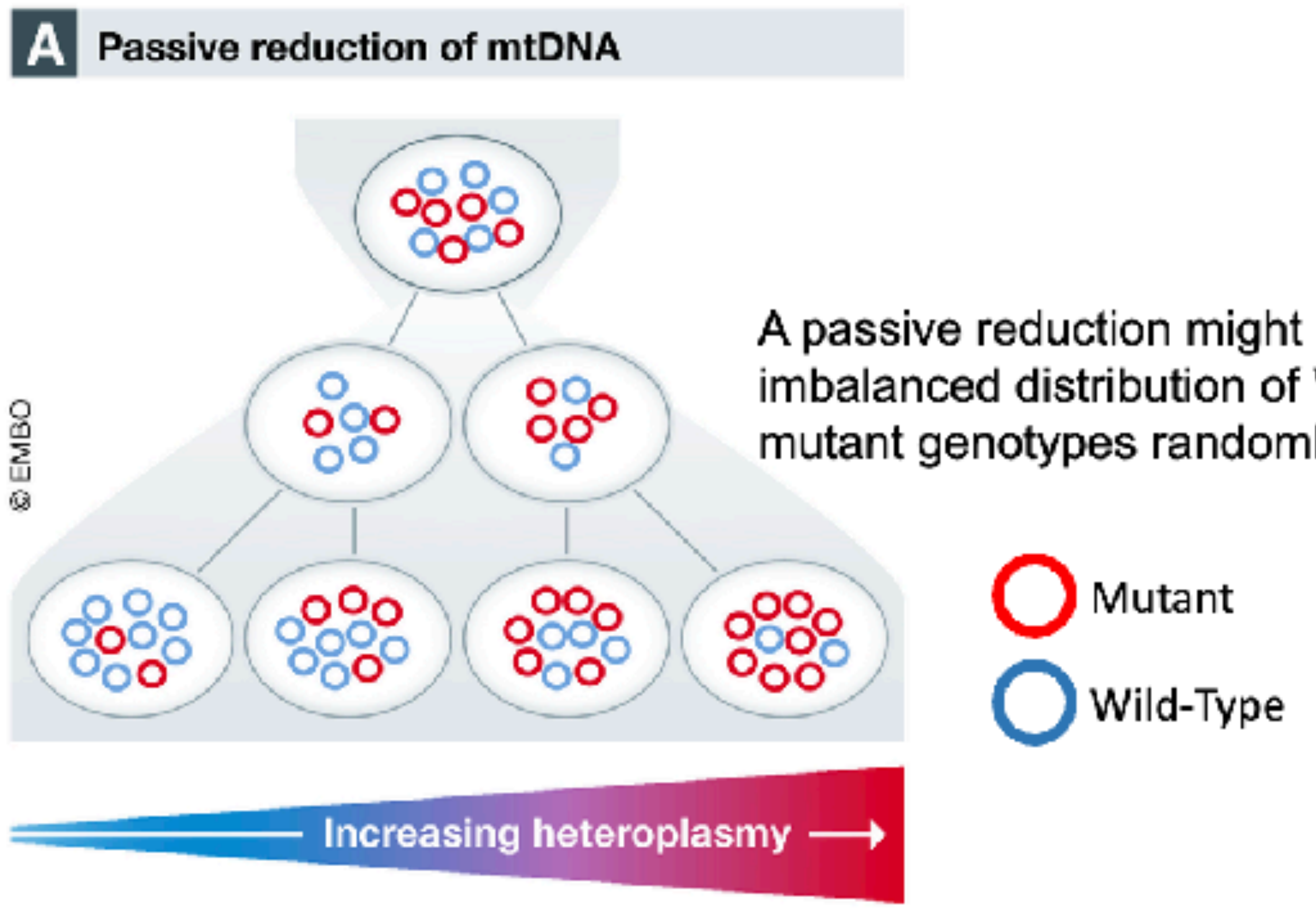


Fan …wallace, 2008

The genetic bottleneck

The "**genetic bottleneck**" refers to the effect of natural fluctuations in mtDNA copy number during germline development, embryogenesis, and subsequent development.

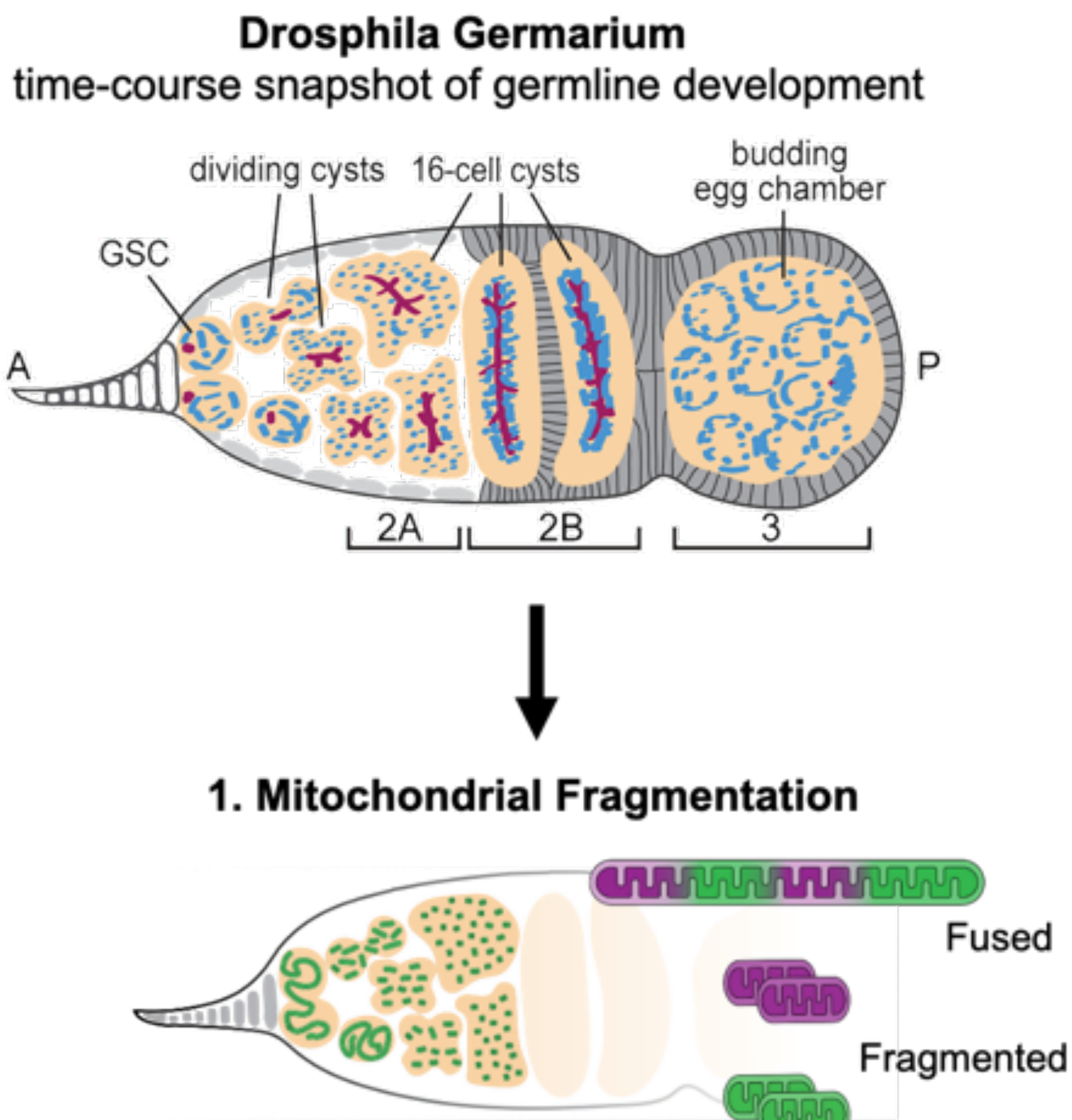
A genetic bottleneck doesn't involve an "active" selection process; rather, it is a **passive mechanism**.

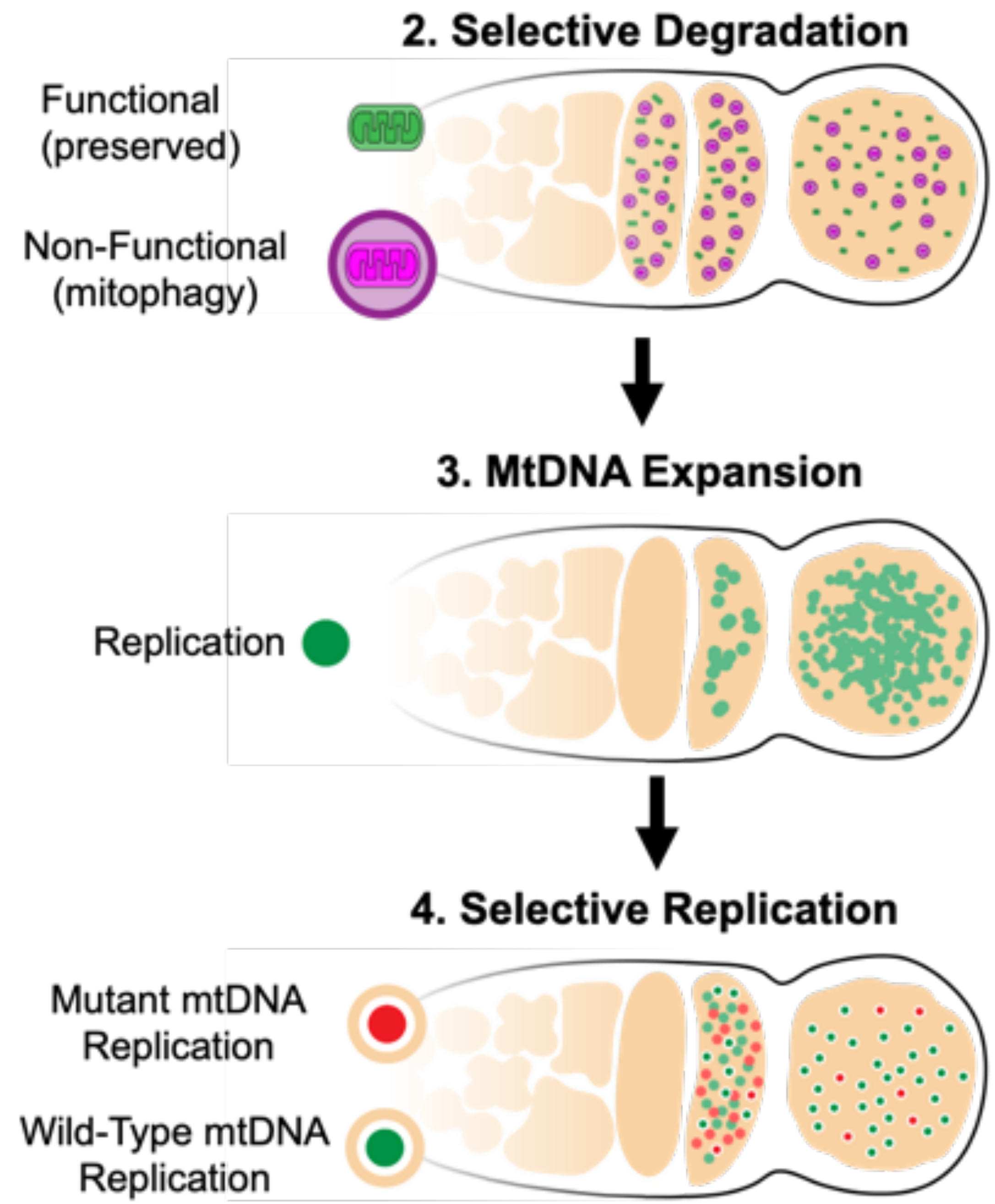


A passive reduction might lead to an imbalanced distribution of WT and mutant genotypes randomly.

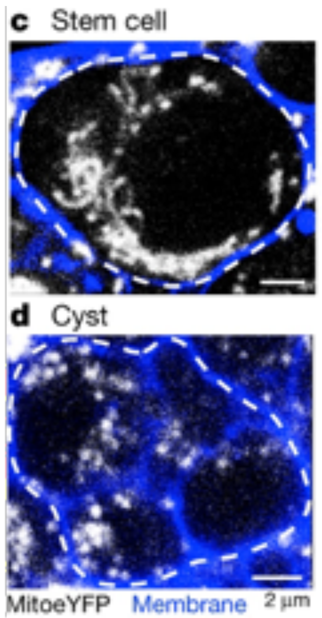
Nissanka and Moraes, EMBO Reports, 2020

Purifying Selection of Mutant MtDNA in the Germline



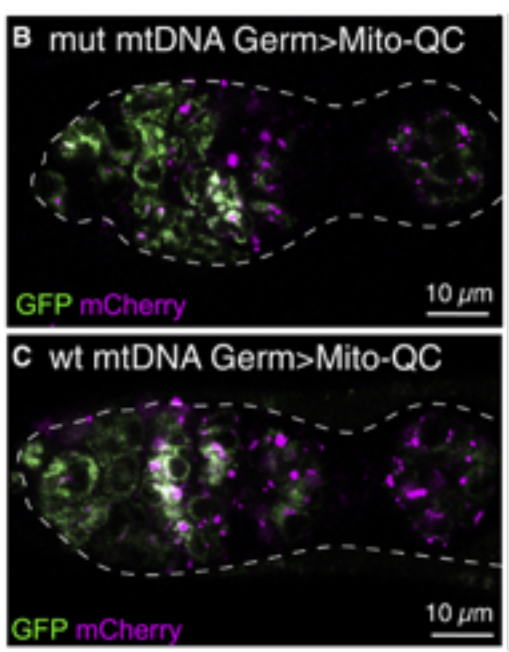


Purifying Selection of Mutant MtDNA in the Germline



1. Mitochondrial Fragmentation

Lieber T, Jeedigunta SP, Palozzi JM, Lehmann R, Hurd TR. Nature. 2019

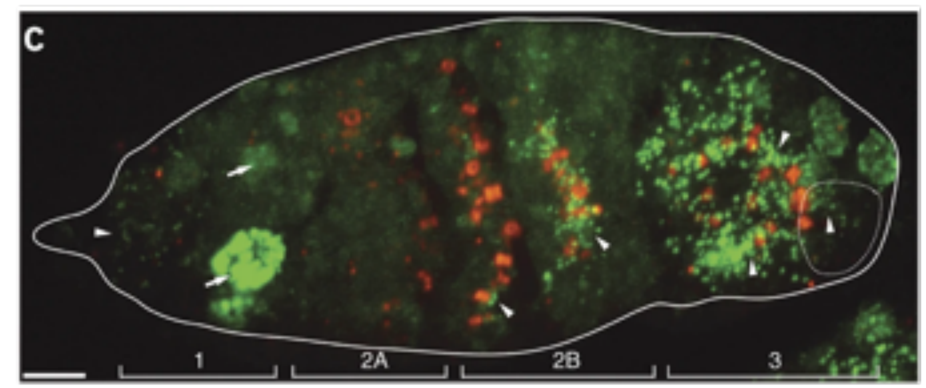


2. Selective Degradation

Palozzi JM, Jeedigunta SP, Minenkova AV, Monteiro VL, Thompson ZS, Lieber T, Hurd TR. Cell Metab. 2022

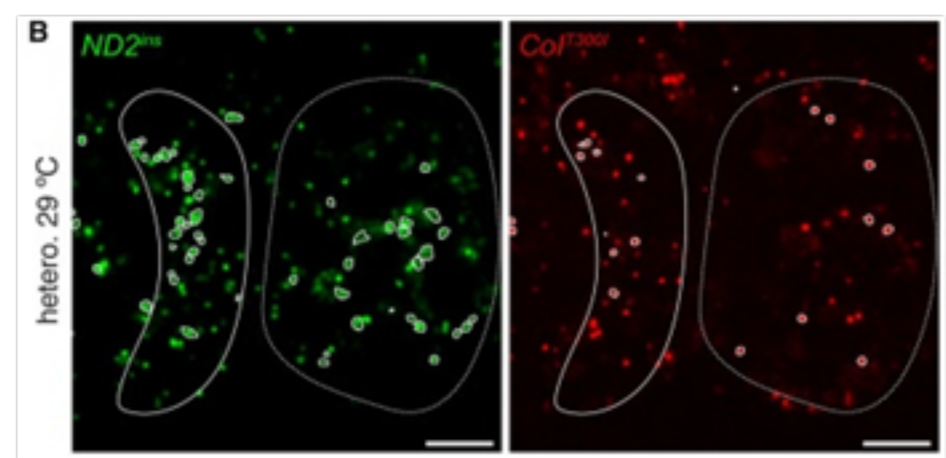
3. MtDNA Expansion

Hill JH, Chen Z, Xu H. Nat Genet. 2014



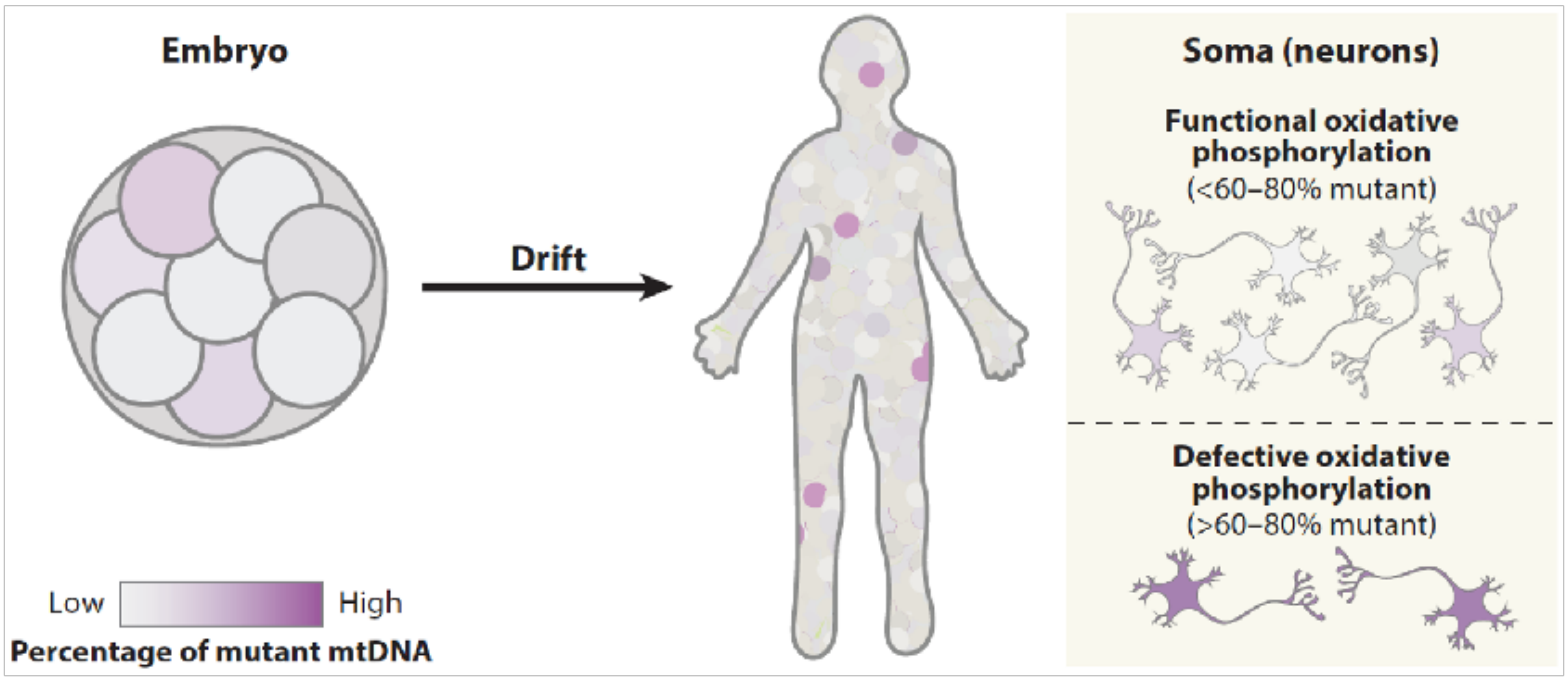
4. Selective Replication

Zhang C, Chen Z, Ma H, Xu H. Cell Rep. 2025



How about the soma ?

One fertilized oocyte will build an entire new organism starting from the same pool of mtDNA. Each tissue rely on mitochondria differently and express different compendium of proteins. Mitochondrial DNA selection can act differently and thereby our body is a mosaic of different mtDNA genotypes.



Jeedigunta SP, Minenkova AV, Palozzi JM, Hurd TR, Annu. Rev. Genom. Hum. Genetic. 2021

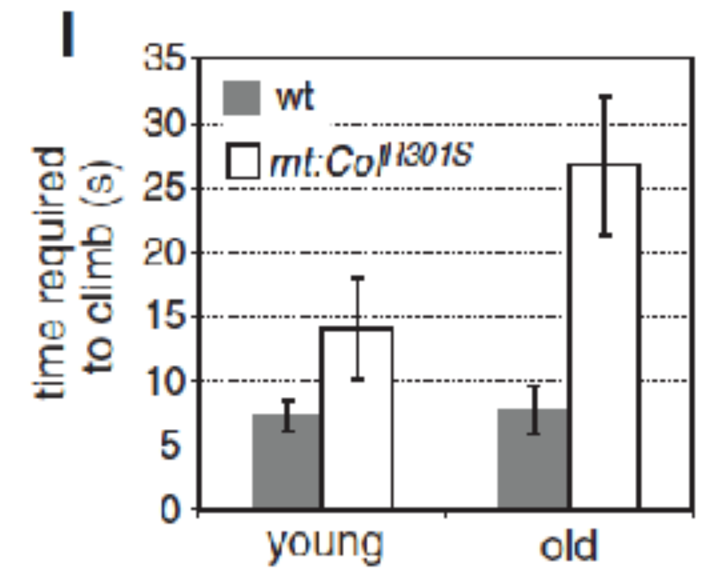
Challenges in manipulating mtDNA

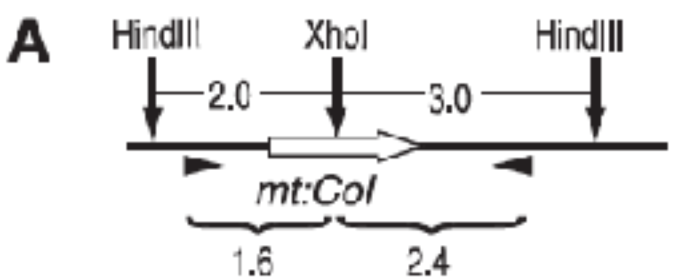
- The inner membrane is negatively charged
- No RNA or DNA import
- No CRISPR targeting
- No DSB repair

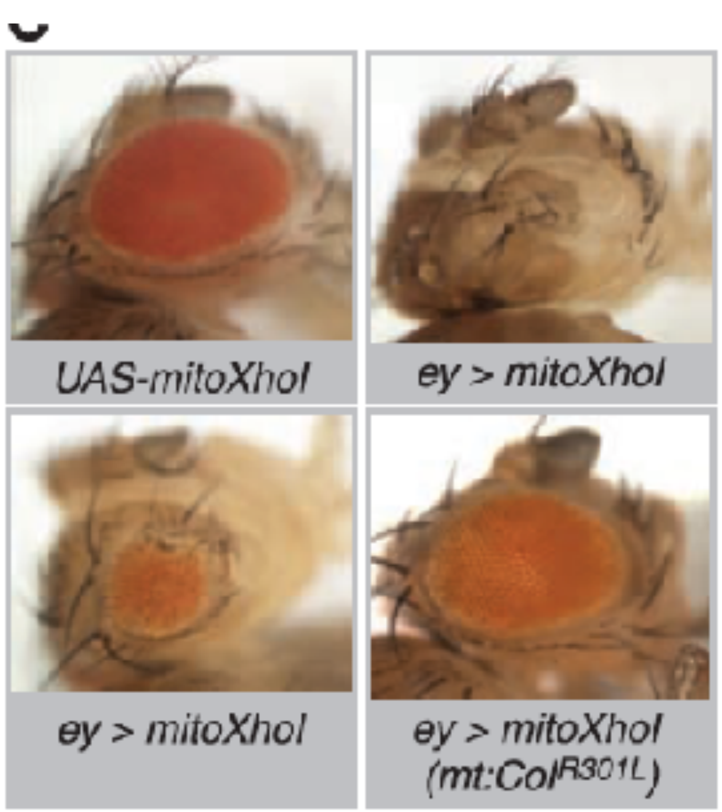
Manipulating the Metazoan Mitochondrial Genome with Targeted Restriction Enzymes

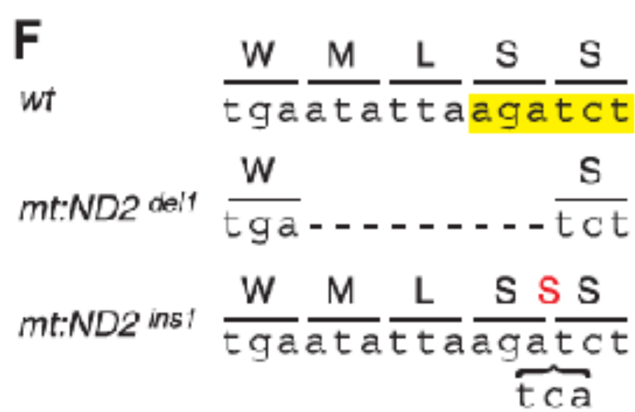
Hong Xu, Steven Z. DeLuca, Patrick H. O'Farrell*

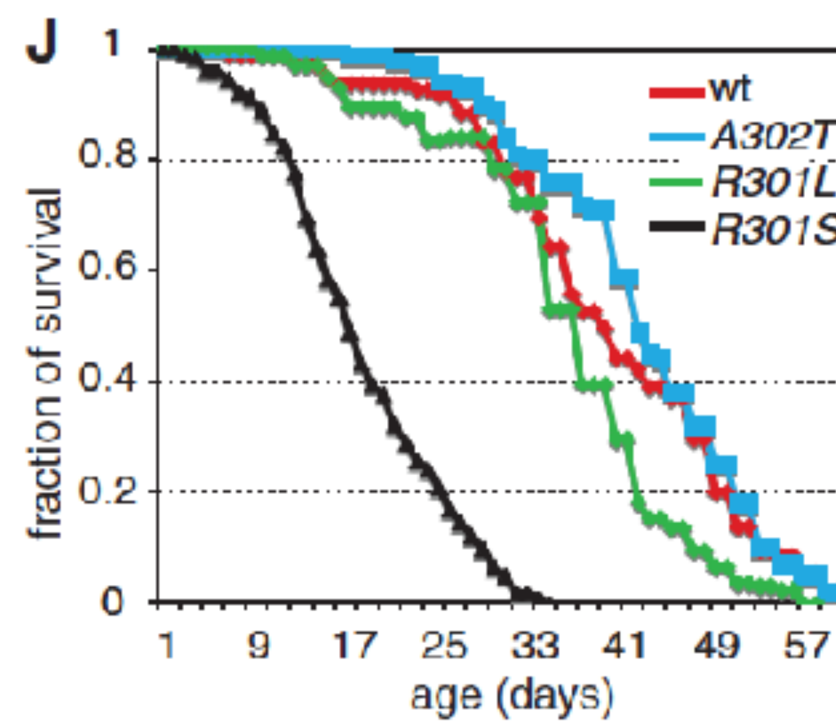
www.sciencemag.org SCIENCE VOL 321 25 JULY 2008



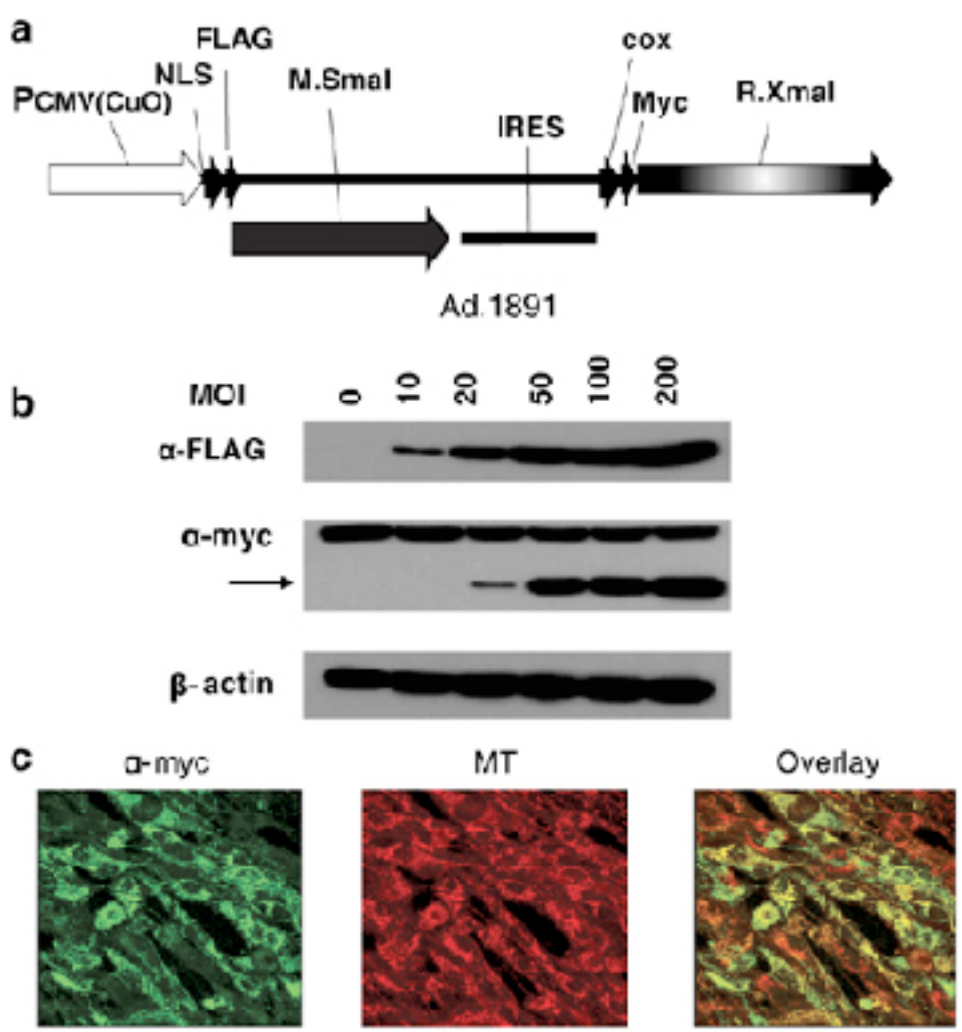


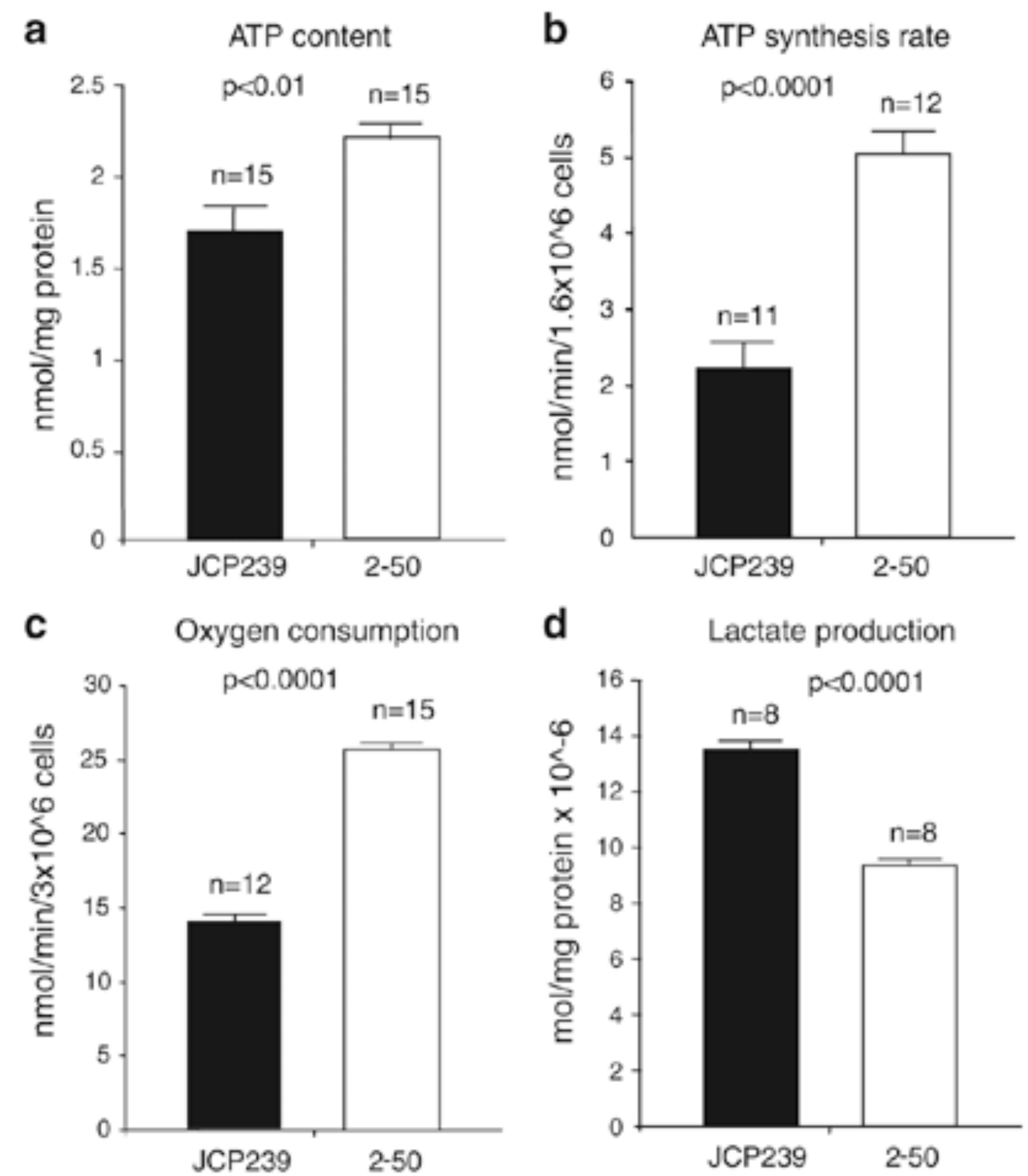






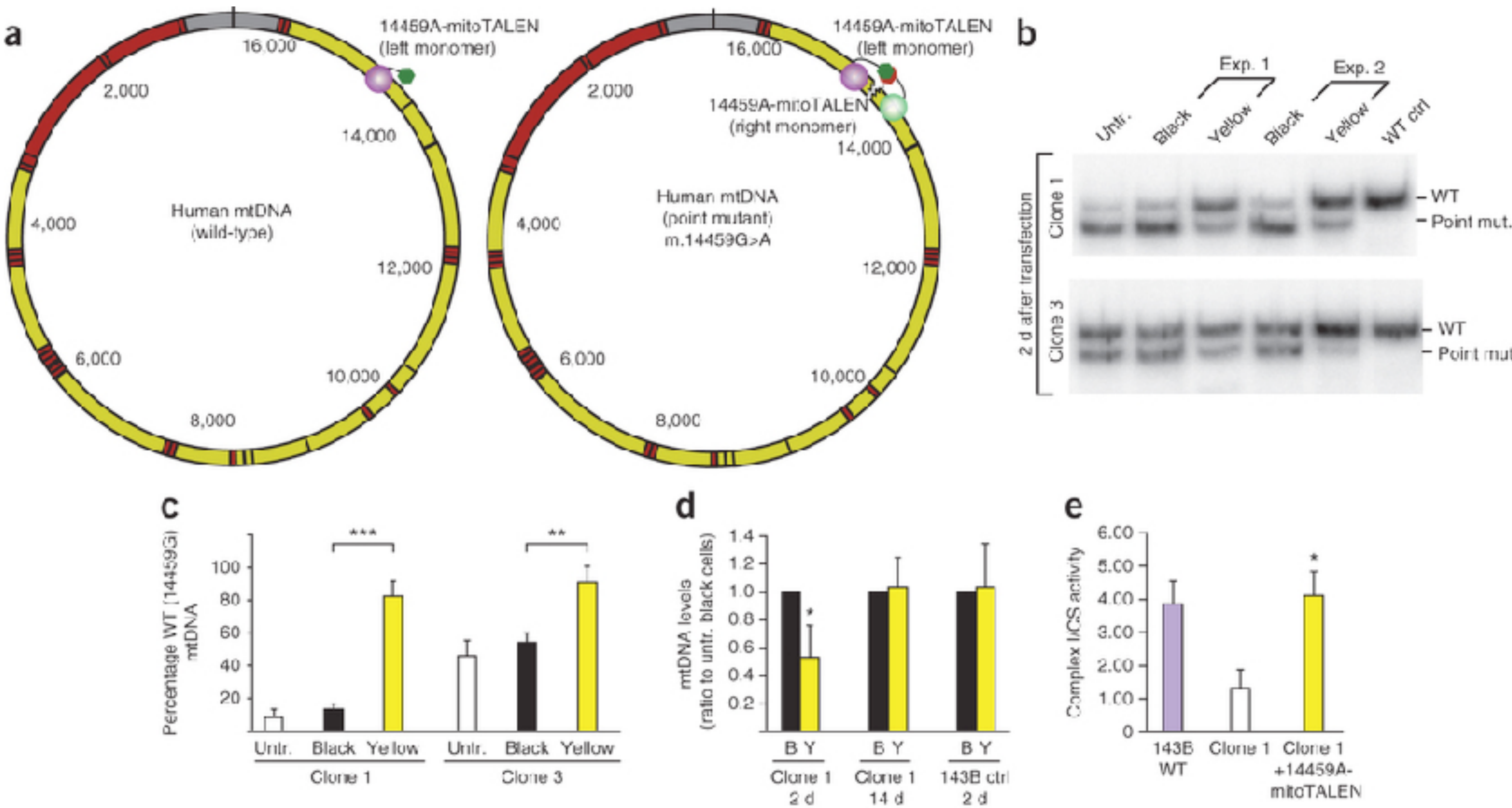
Selective elimination of mutant mitochondrial genomes with restriction enzymes





Alexeyev et al., 2008

Specific elimination of mutant mitochondrial genomes by mitoTALENs



Backman et al., 2013

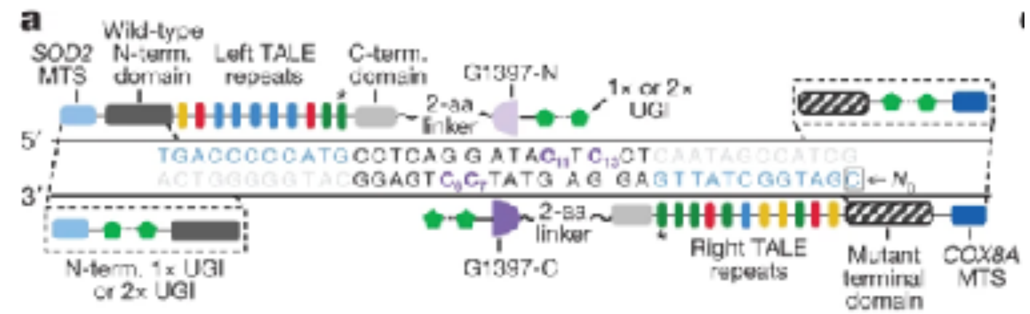
Base editors to introduce point mutations in mtDNA

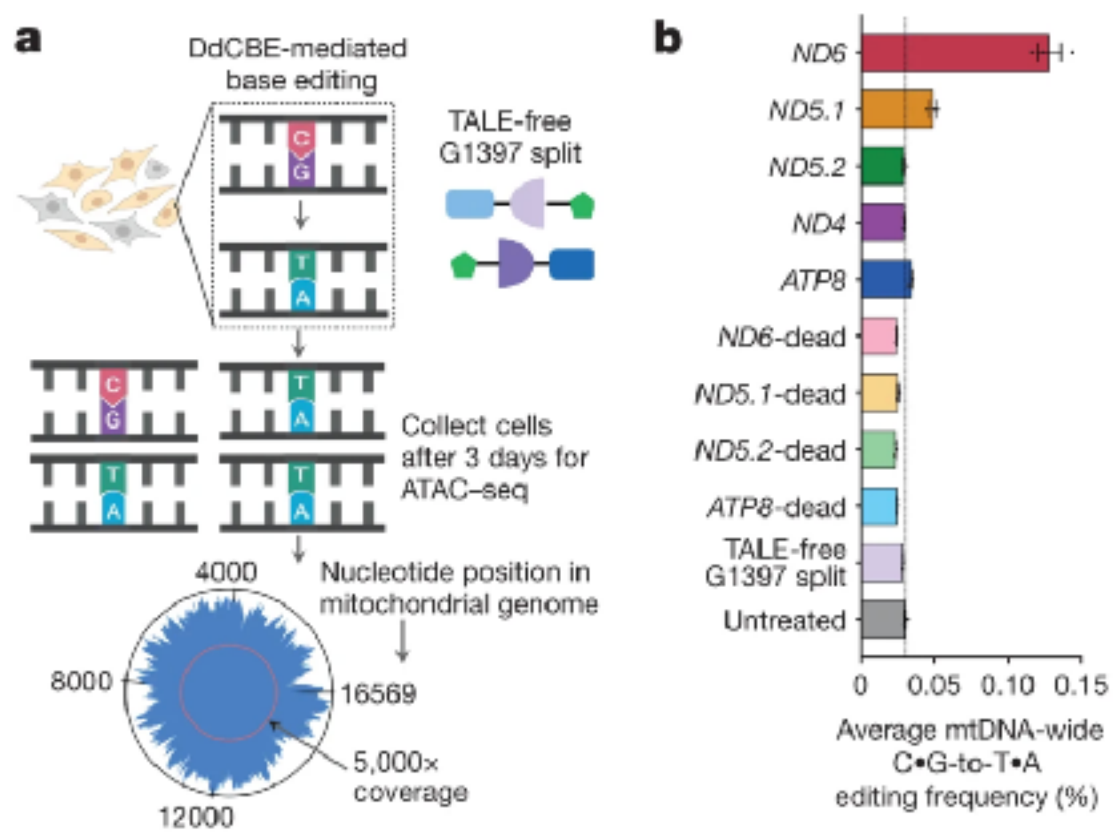
Article | Published: 08 July 2020

A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing

Beverly Y. Mok, Marcos H. de Moraes, Jun Zeng, Dustin E. Bosch, Anna V. Kotrys, Aditya Raguram, FoSheng Hsu, Matthew C. Radey, S. Brook Peterson, Vamsi K. Mootha, Joseph D. Mougous & David R. Liu

Nature 583, 631–637 (2020) | Cite this article





nature biomedical engineering

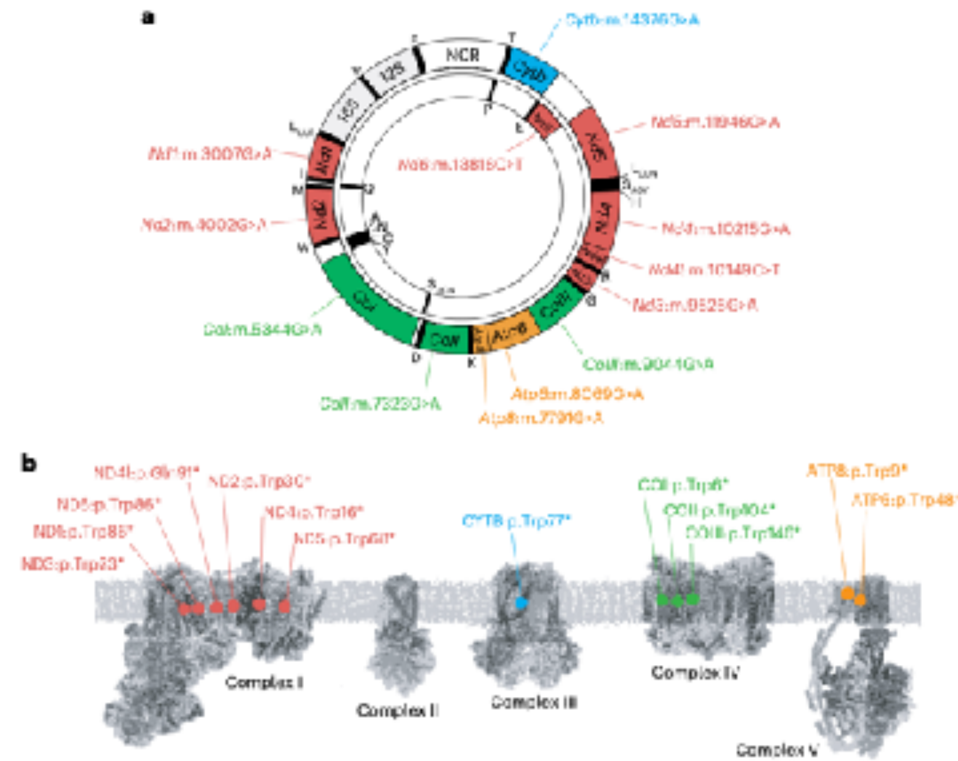
Article https://doi.org/10.1038/s41551-022-00968-1

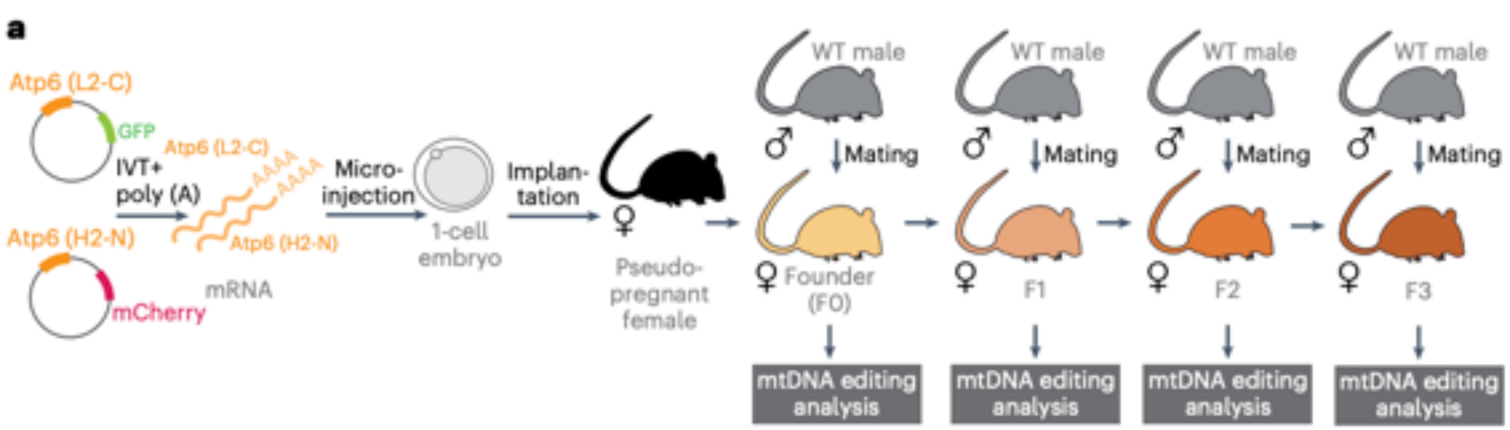
A library of base editors for the precise ablation of all protein-coding genes in the mouse mitochondrial genome

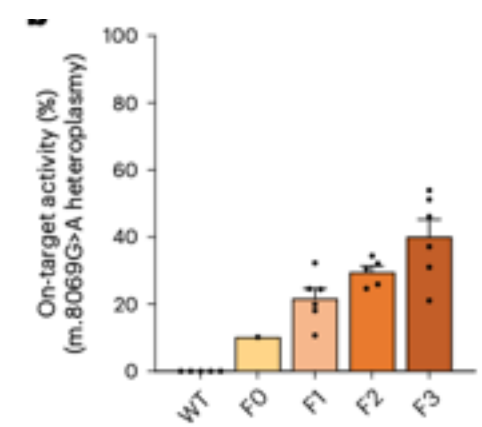
Received: 1 June 2022

Accepted: 20 October 2022

Pedro Silva-Pinheiro, Christian D. Mutti, Lindsey Van Haute, Christopher A. Powell, Pavel A. Nash, Keira Turner & Michal Minczuk

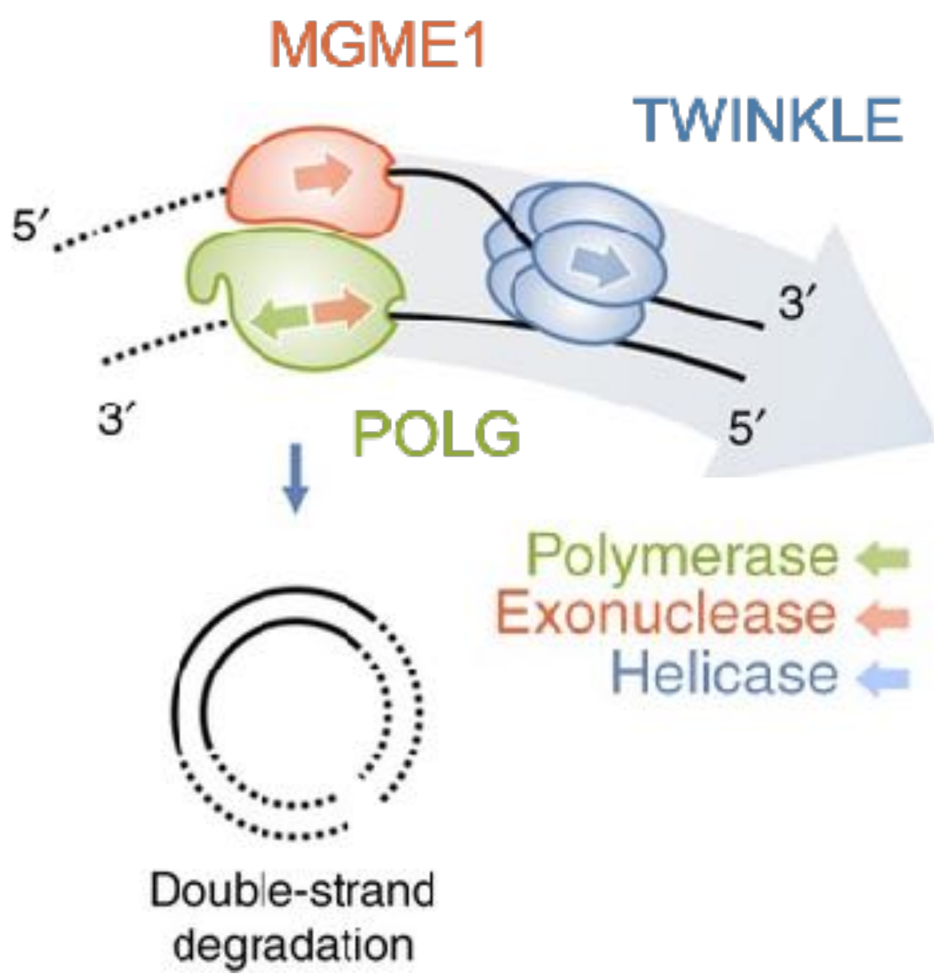


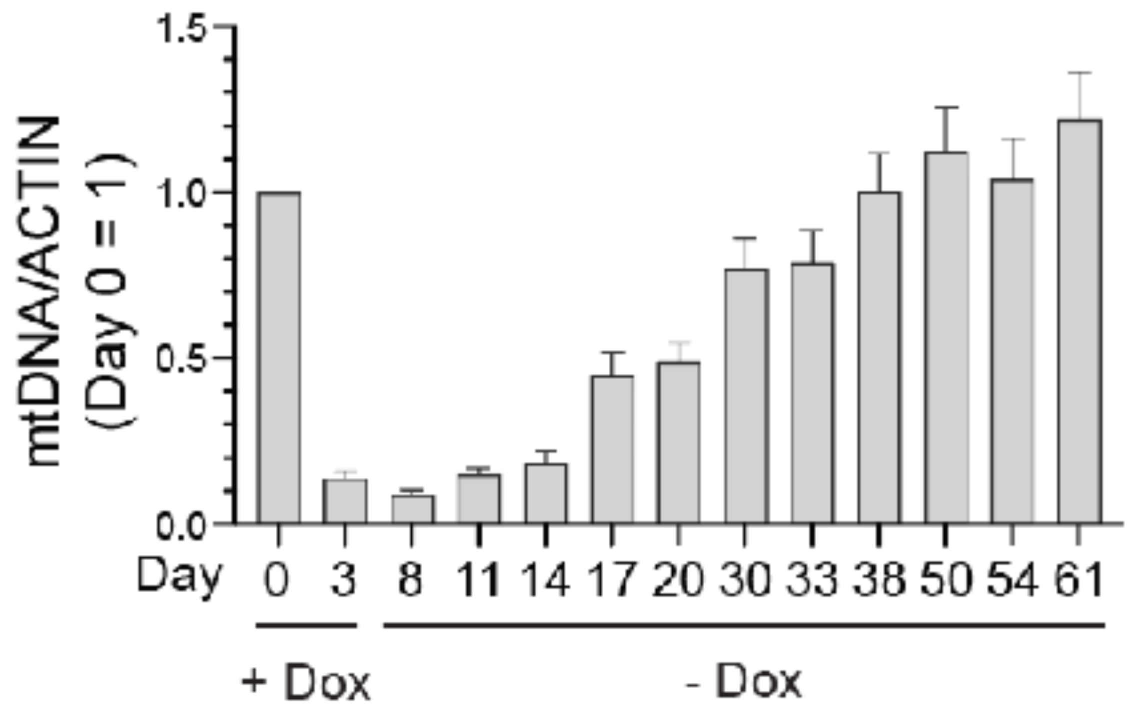




Major challenges to engineering mtDNA deletions

1. No gene targetting in the mitochondria
2. No DSB repair in mitochondrial
3. Cleaved mtDNA is rapidly degraded

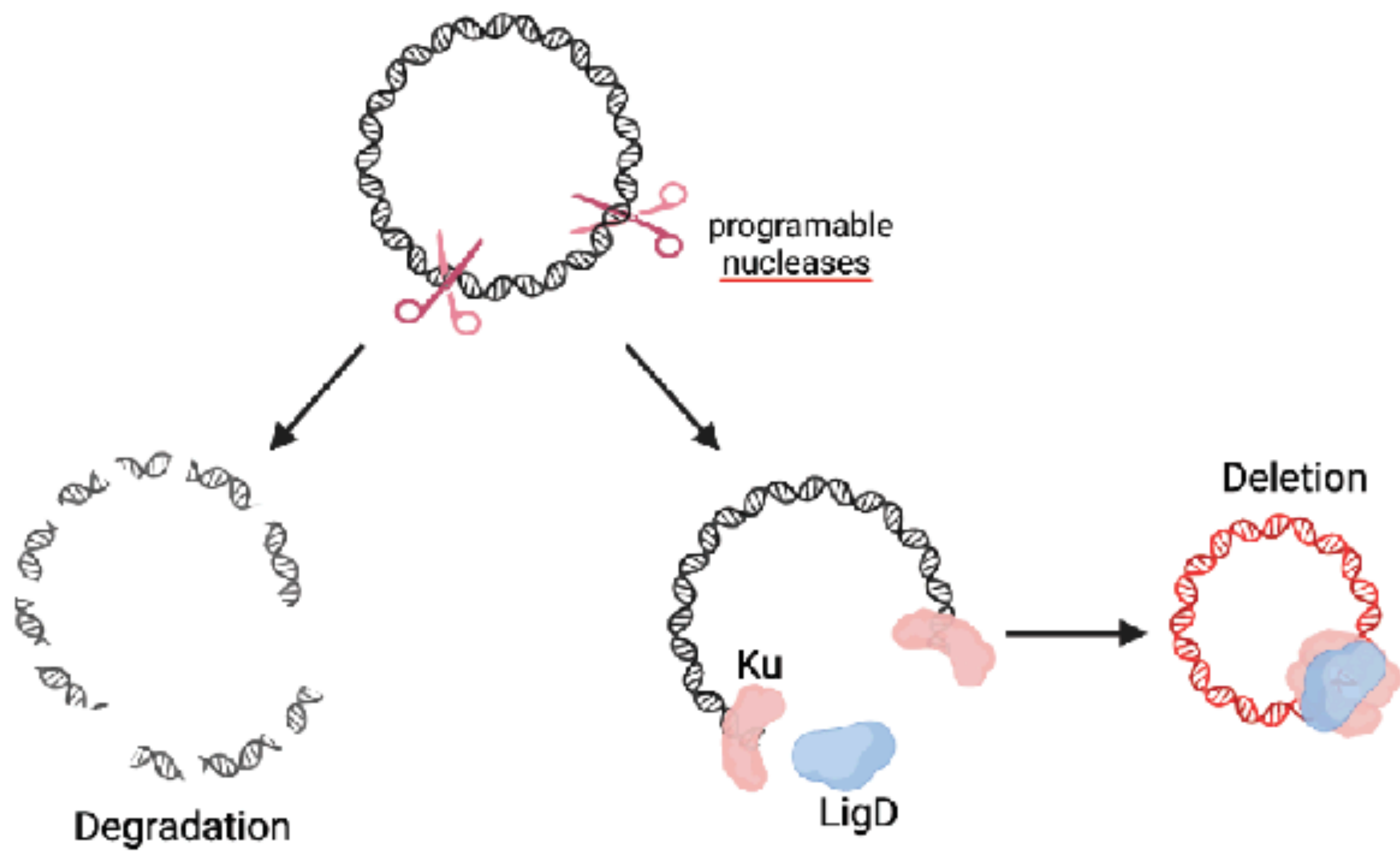


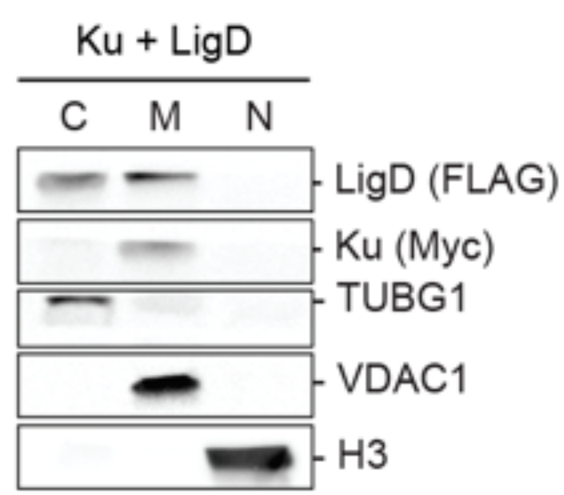


Dox inducible mito-ApaLI

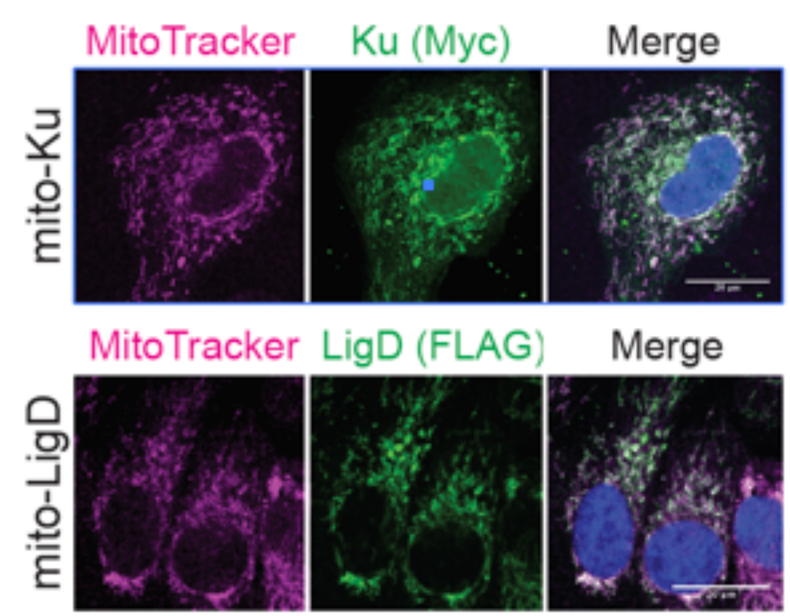
Nissanka, et al. 2018. PMID: 29950568 Nicholls, et al. 2014. PMID: 24986917 Peeva, et al. 2018. PMID: 29712893

Reconstituting end-joining (*Mycobacterium*) in human mitochondria





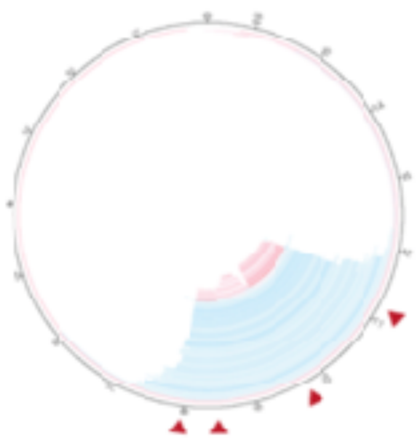
RPE cells



Fu, Land, Kavlashvili, PMID: 39463974

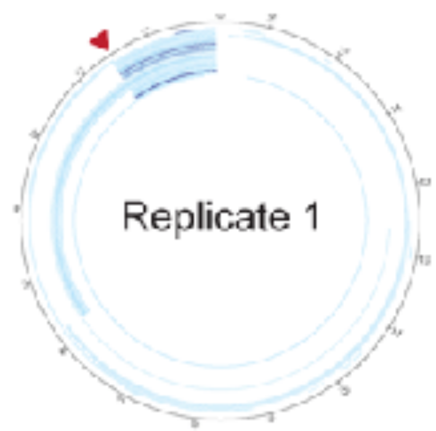
Modeling different deletions in various cell types

Large



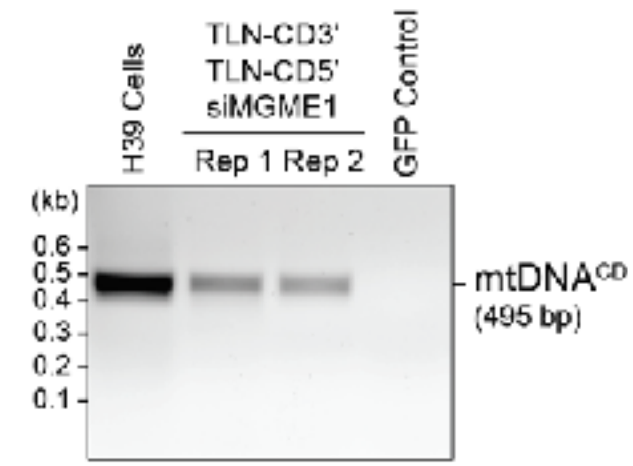
mito-ScaI

Small



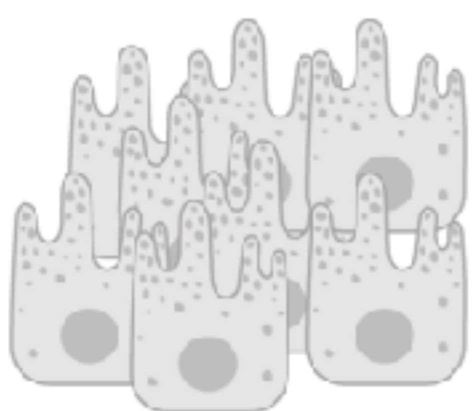
mito-ApaLI

Common deletion



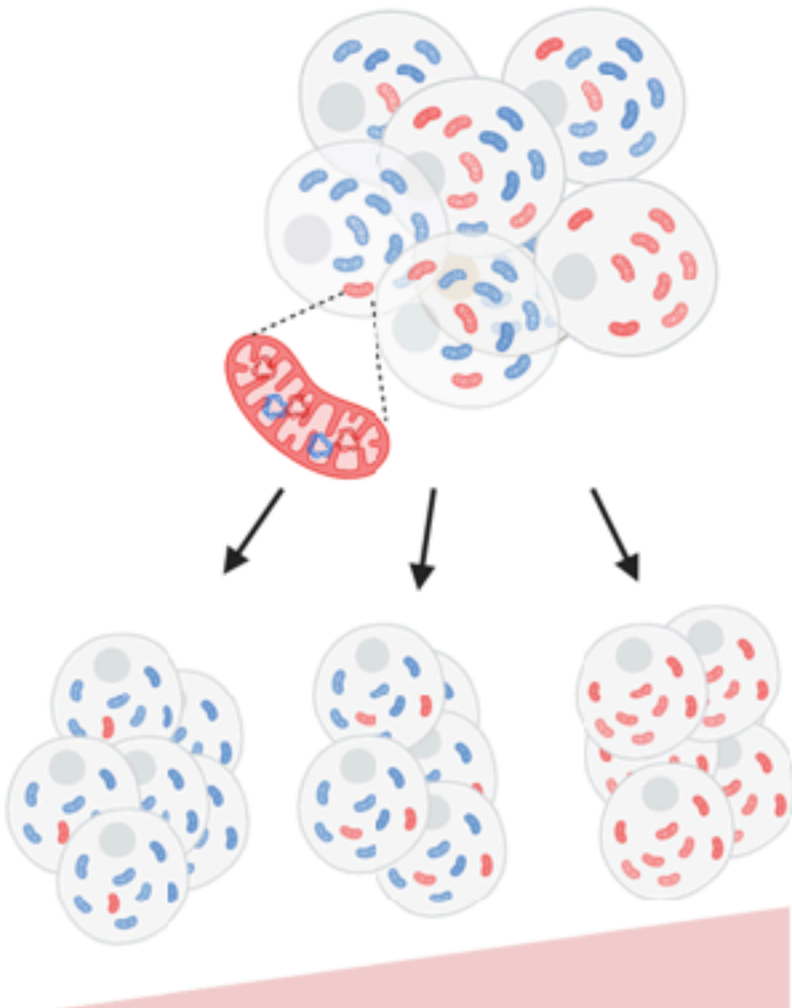
mito-TALENs

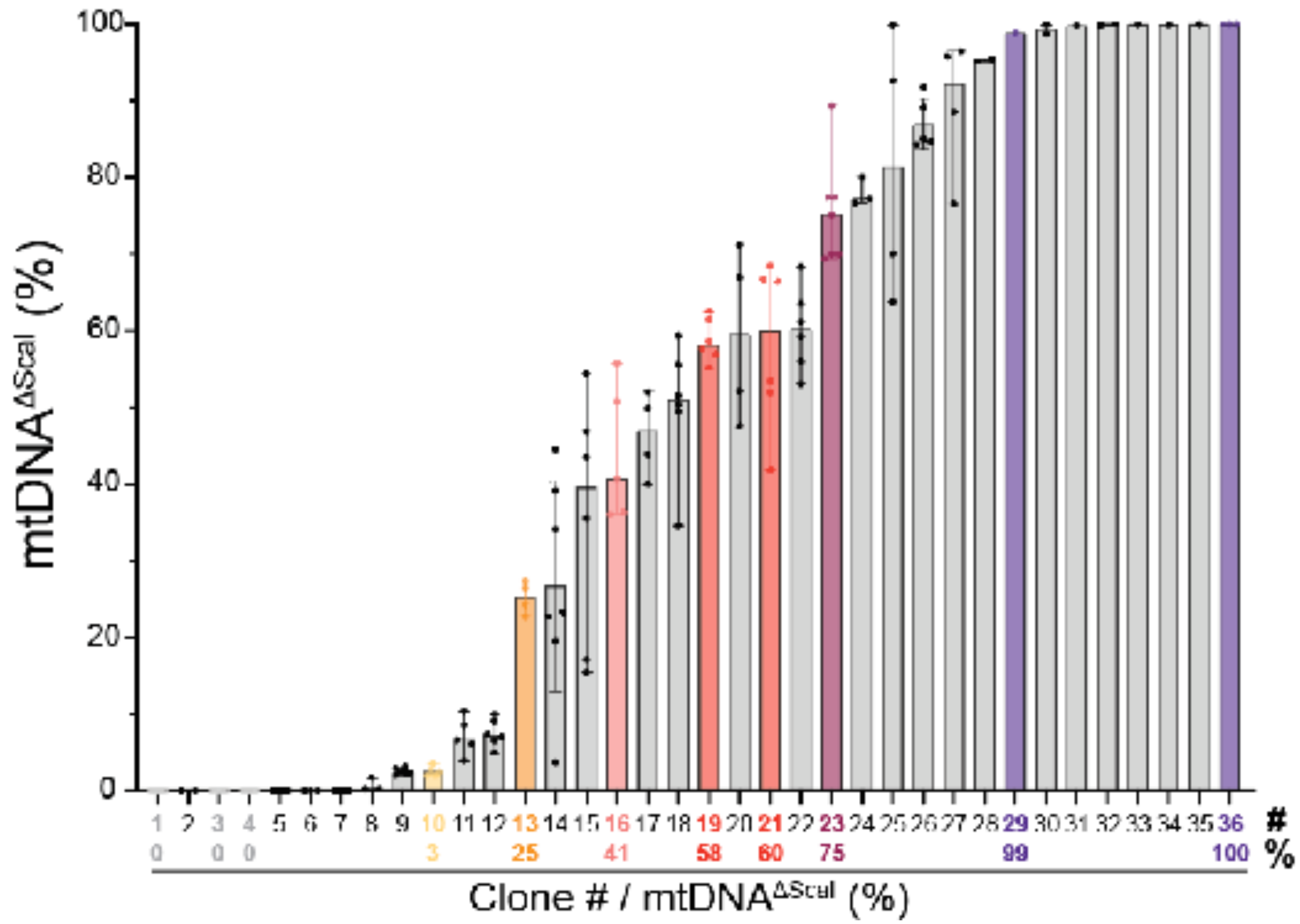
Retinal Pigmental Epithelial (RPE)



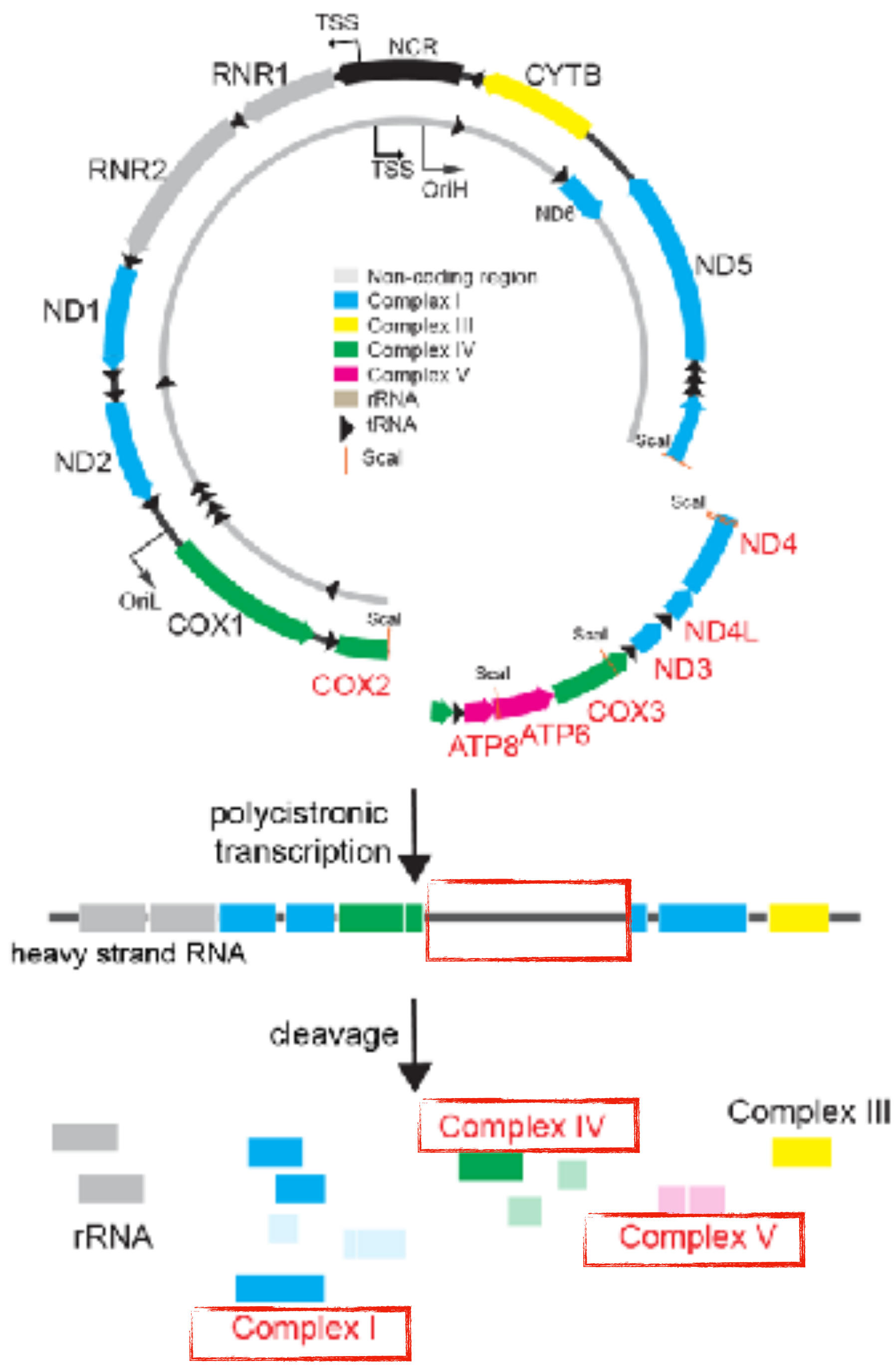
iPSCs

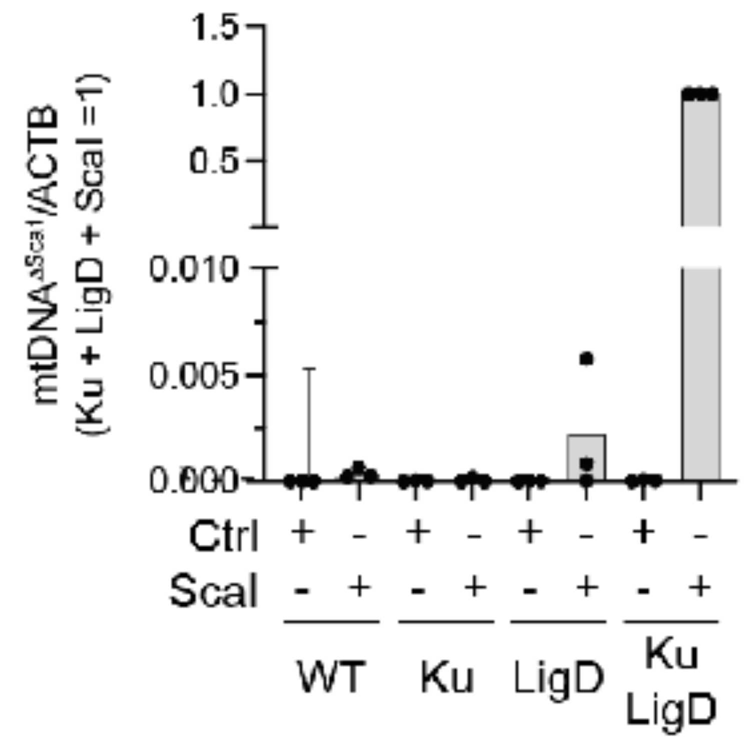
A panel of cell lines carrying mtDNA deletion with full spectrum of heteroplyasmy





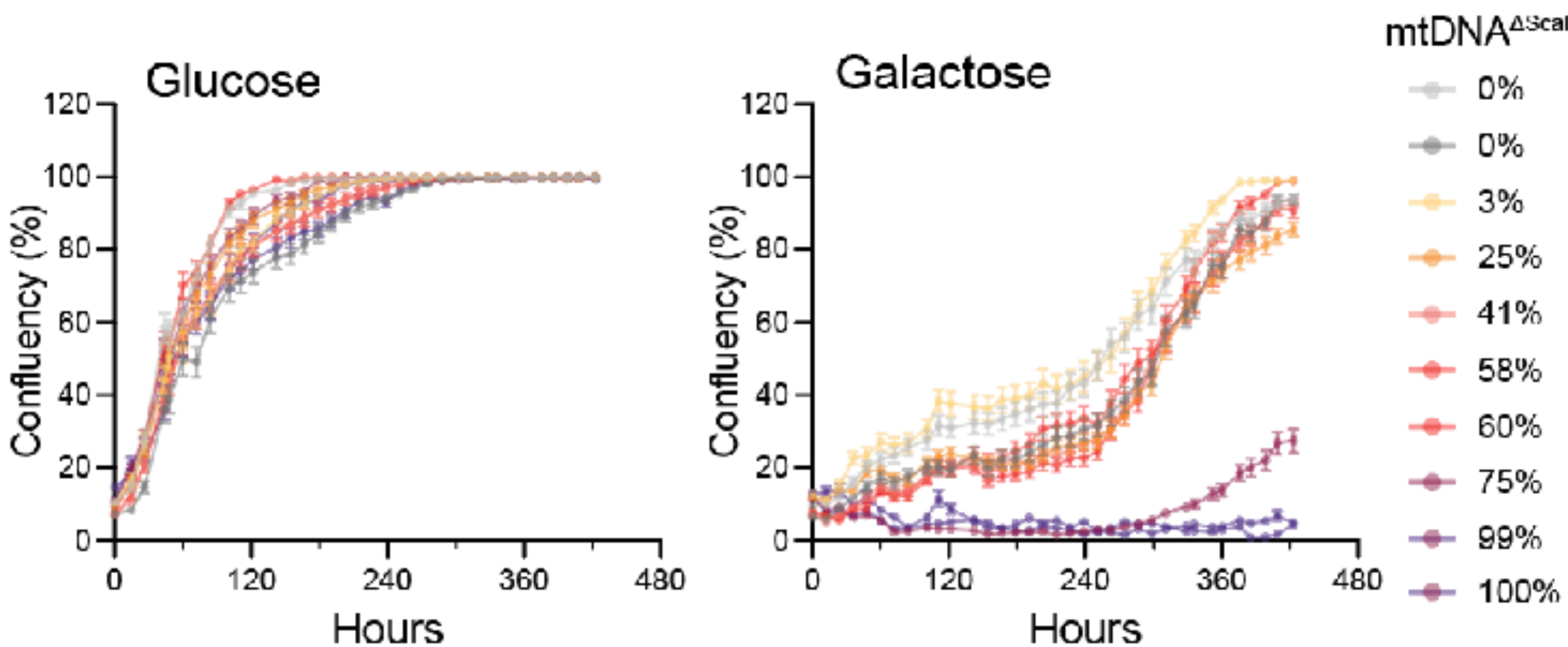
ScaI cleavage deletes a 3.9 Kb region (6 proteins and 3 tRNAs)



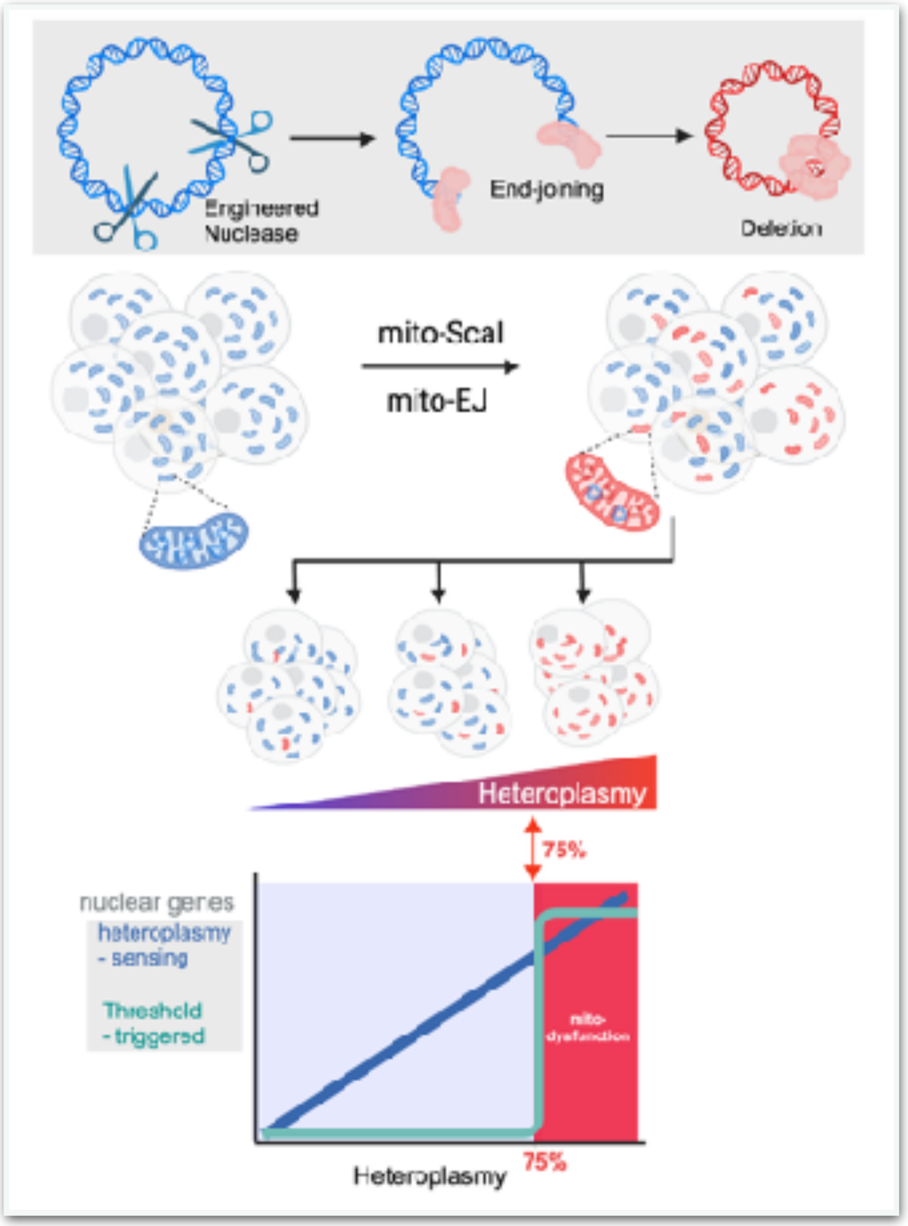


**cellular heterogeneity
(Various heteroplasmy)

Forcing cells to rely on OXPHOS (Galactose-media) impairs cellular growth



Summary



- Established a genetic tool to engineer precise mtDNA deletions
- We engineered a panel of cell lines to probe the impact of mtDNA deletions metabolically and at the molecular and cellular levels
- ~75% heteroplasmy is the threshold for large deletion to trigger cellular dysfunction.
- Two sensing modes: 1- threshold-triggered and 2- heteroplasmy-sensing

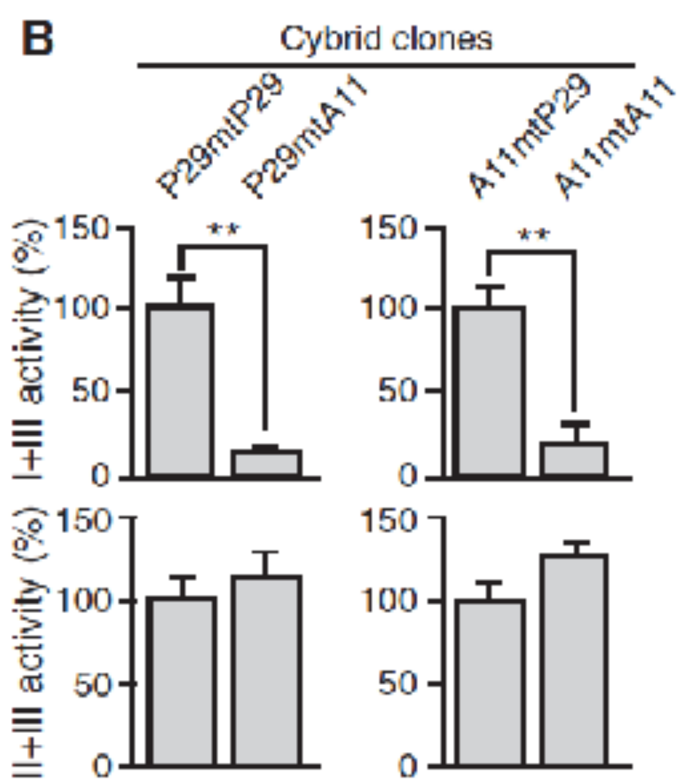
mtDNA mutations and tumor

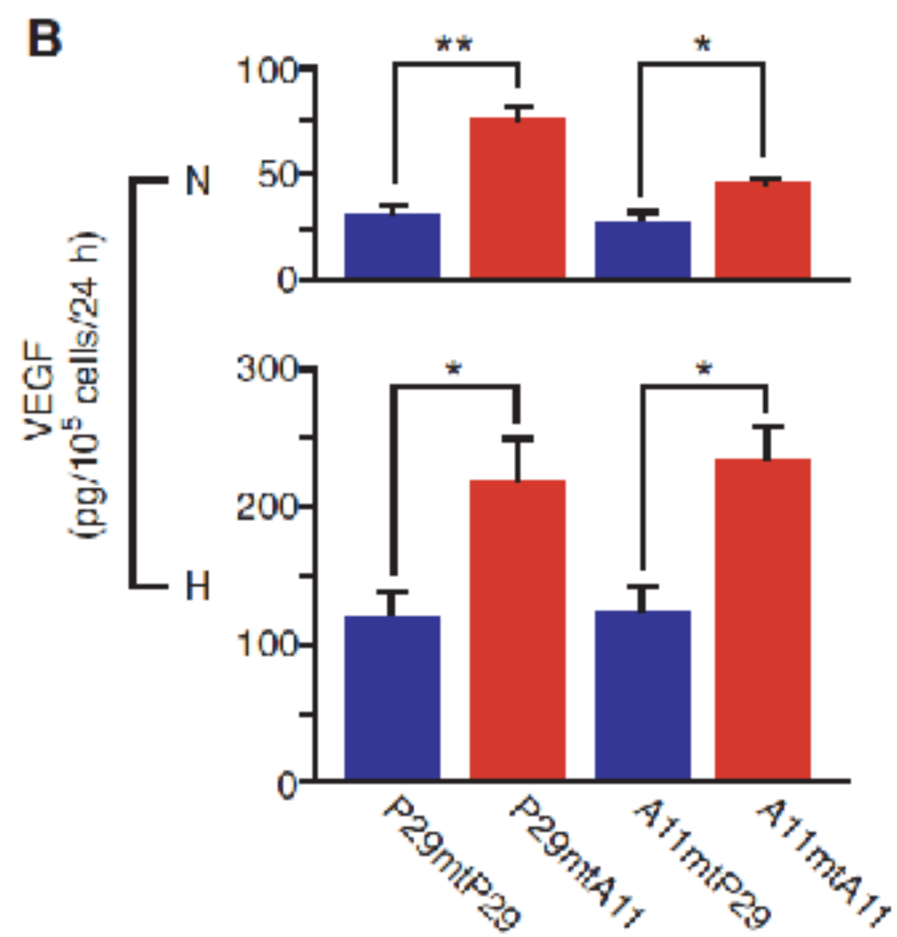
ROS-Generating Mitochondrial DNA Mutations Can Regulate Tumor Cell Metastasis

Kaori Ishikawa,[1,2,3]* Keizo Takenaga,[4,5]* Miho Akimoto,[5] Nobuko Koshikawa,[4] Aya Yamaguchi,[1] Hirotake Imanishi,[1] Kazuto Nakada,[1,2] Yoshio Honma,[5] Jun-Ichi Hayashi[1]†

mtDNA conferring high metastatic potential contained G13997A and 13885insC

low metastatic P29 and high metastatic A11 cells

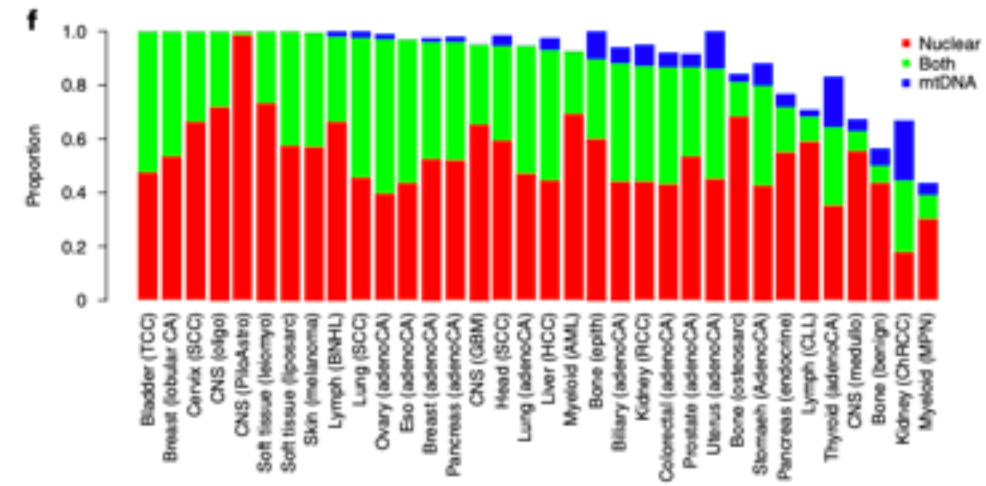


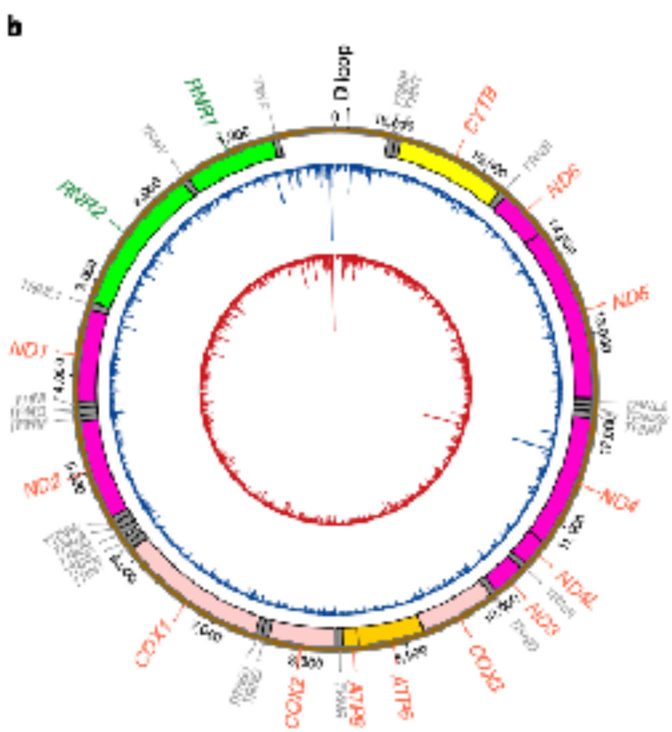


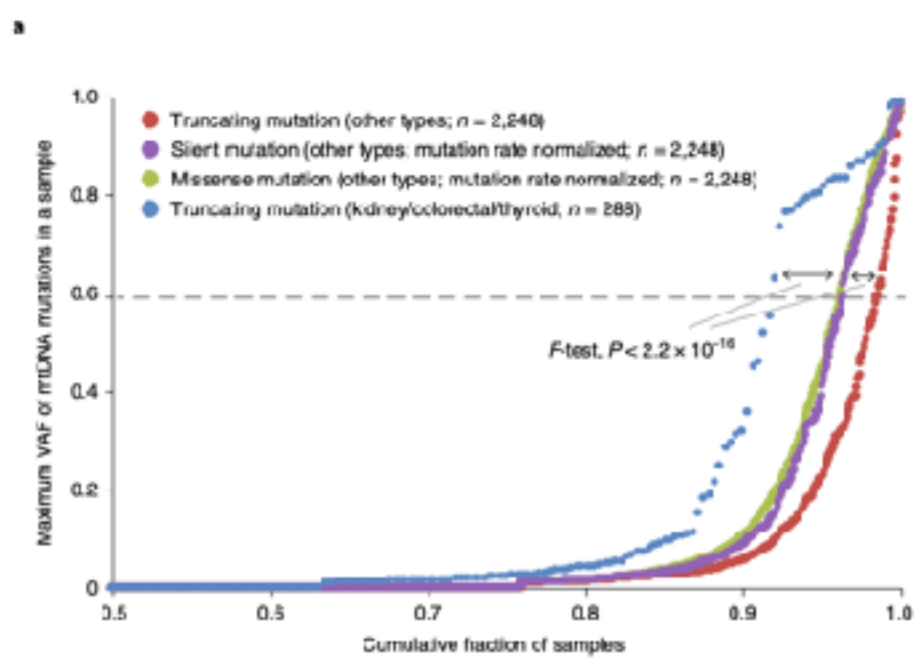
Analysis of cancer genomes identifies frequent mtDNA mutations

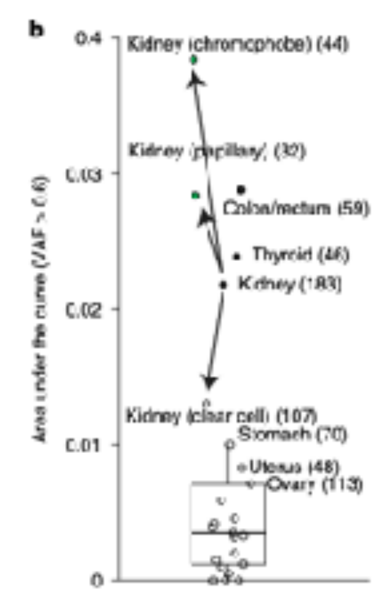
Comprehensive molecular characterization of mitochondrial genomes in human cancers

Yuan Yuan[1,9,20], Young Seok Ju[2,3,9,20], Youngwook Kim[4,5,9,20], Jun Li[1], Yumeng Wang[1,6], Christopher J. Yoon[3], Yang Yang[7], Inigo Martincorena[2], Chad J. Creighton[8], John N. Weinstein[1,9], Yanxun Xu[10], Leng Han[11], Hyung-Lae Kim[12], Hidewaki Nakagawa[13], Keunchil Park[14,15], Peter J. Campbell[2,16], Han Liang[1,5,9] and PCAWG Consortium*

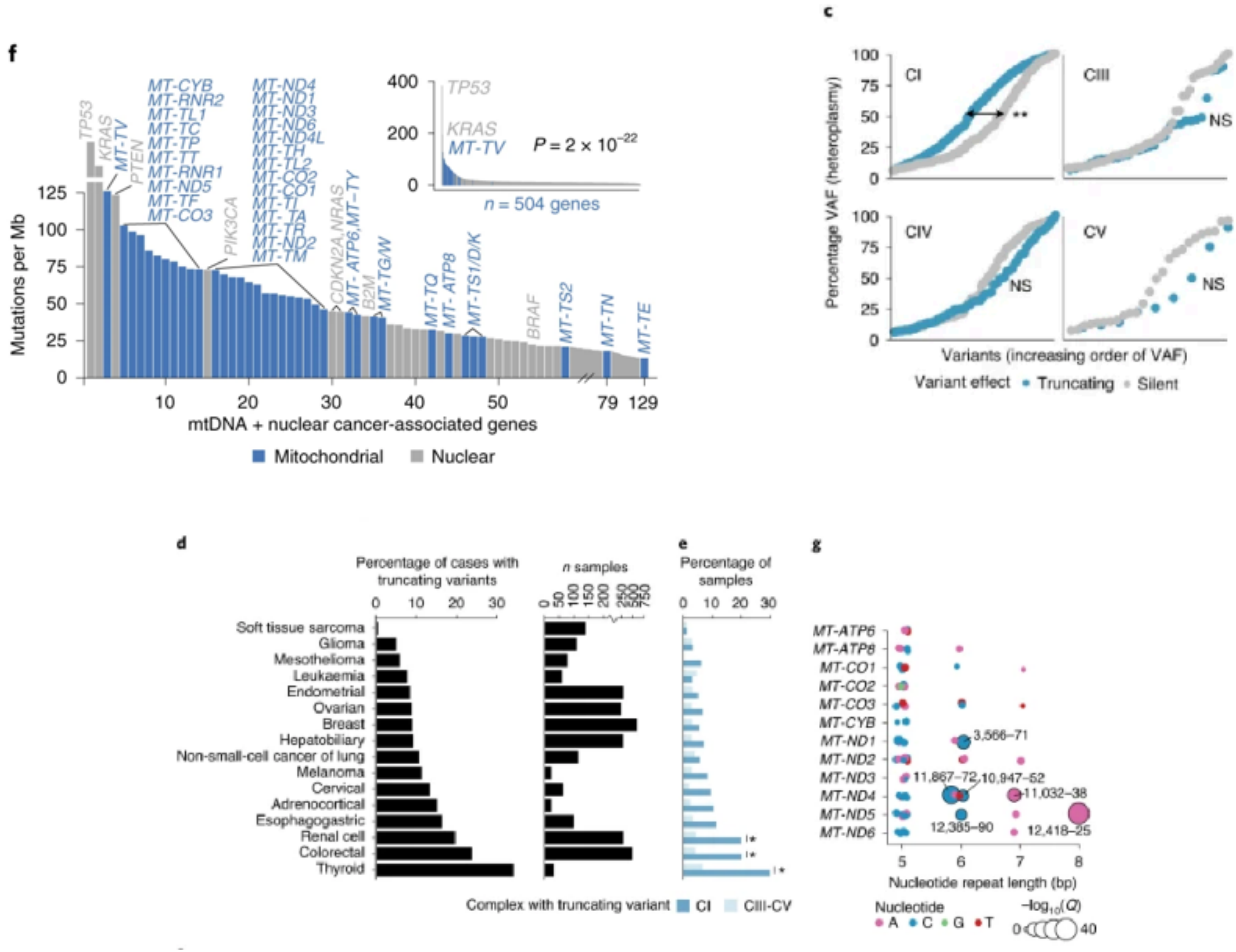








Enrichment of Complex I mutations



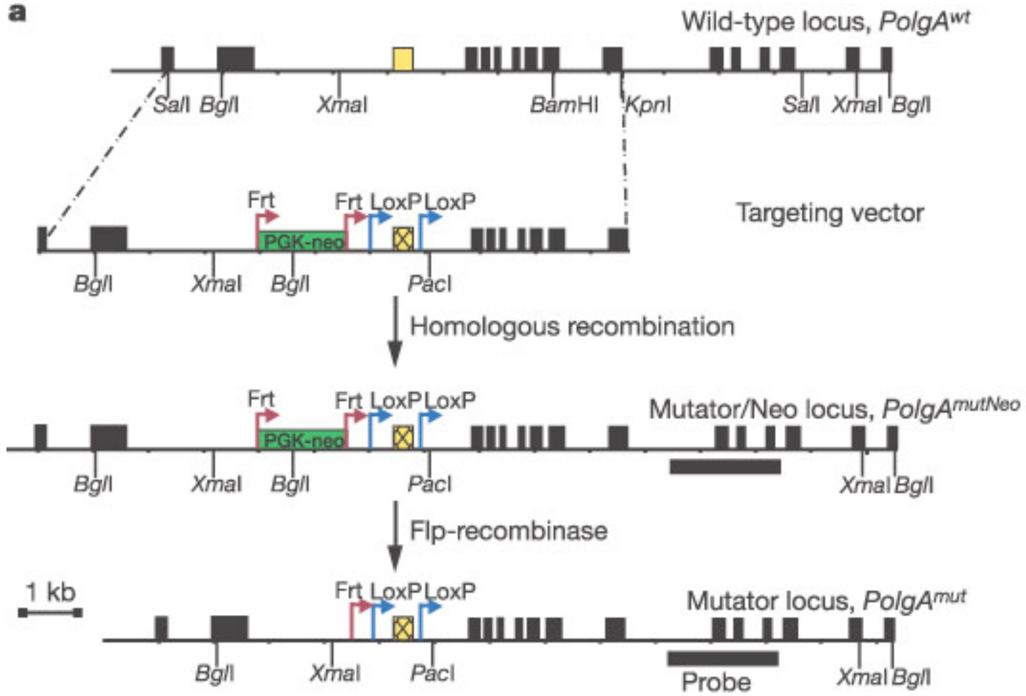
mitochondria and aging … the mutator mouse

Premature ageing in mice expressing defective mitochondrial DNA polymerase

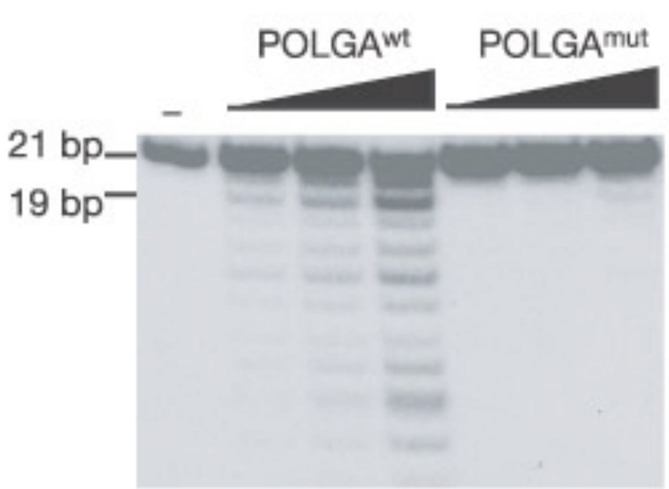
Aleksandra Trifunovic[1,2], Anna Wredenberg[1,2], Maria Falkenberg[1], Johannes N. Spelbrink[3], Anja T. Rovio[3], Carl E. Bruder[4], Mohammad Bohlooly-Y[4], Sebastian Gidlöf[1,2], Anders Oldfors[5], Rolf Wibom[6], Jan Törnell[4], Howard T. Jacobs[3] & Nils-Göran Larsson[1,2]

NATURE | VOL 429 | 27 MAY 2004 | www.nature.com/nature

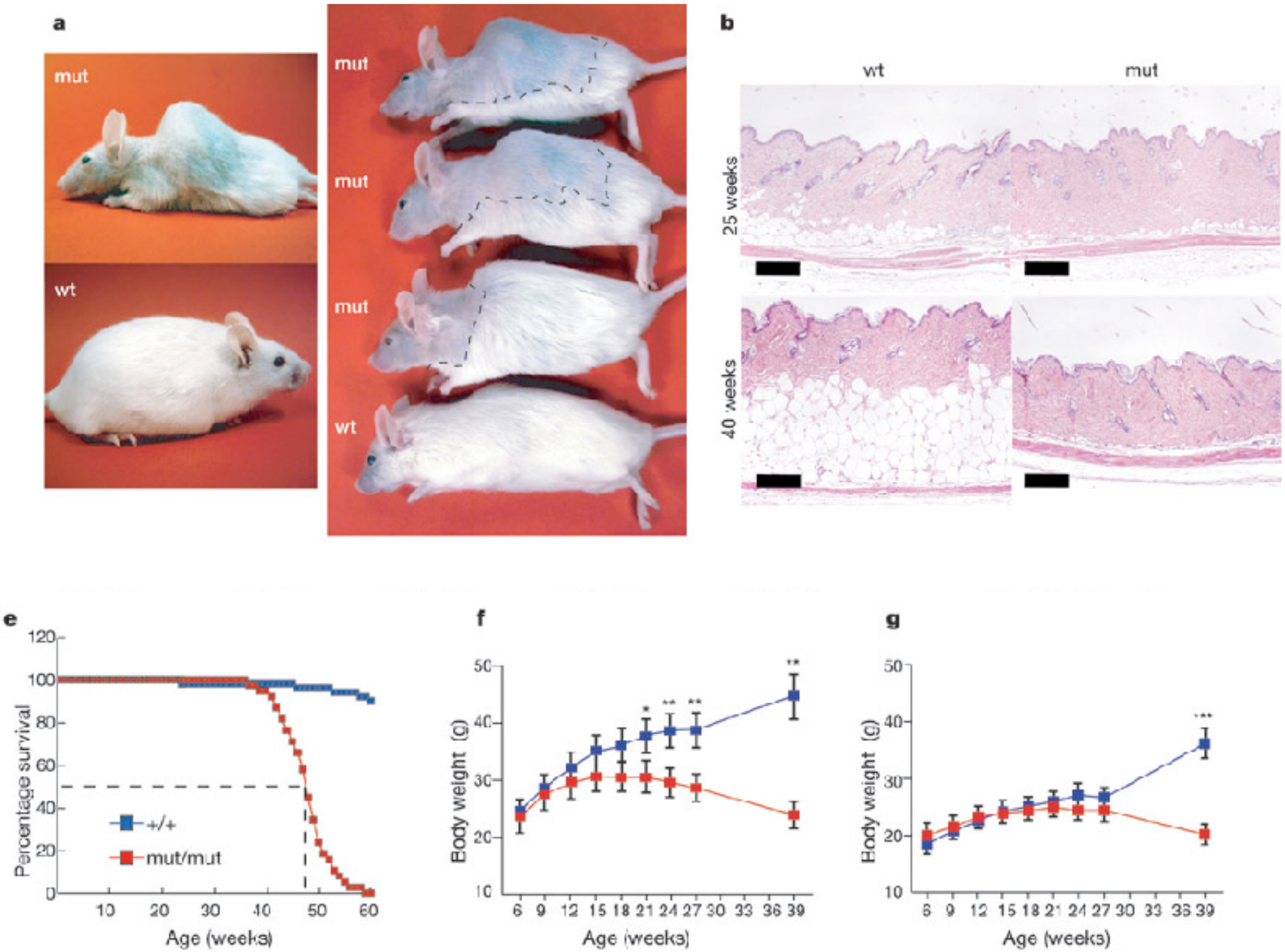
a



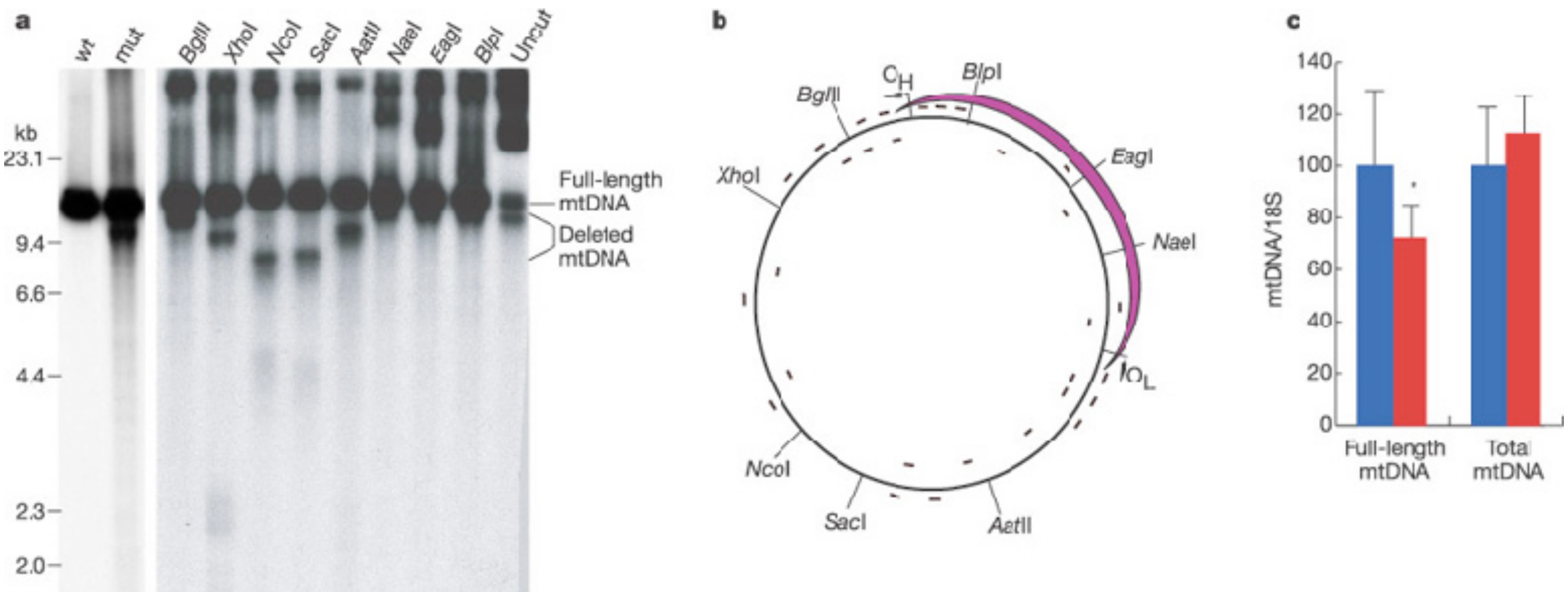
e



Age-related phenotypes observed in mtDNA-mutator mice.



Analysis of mtDNA in the mutator mouse



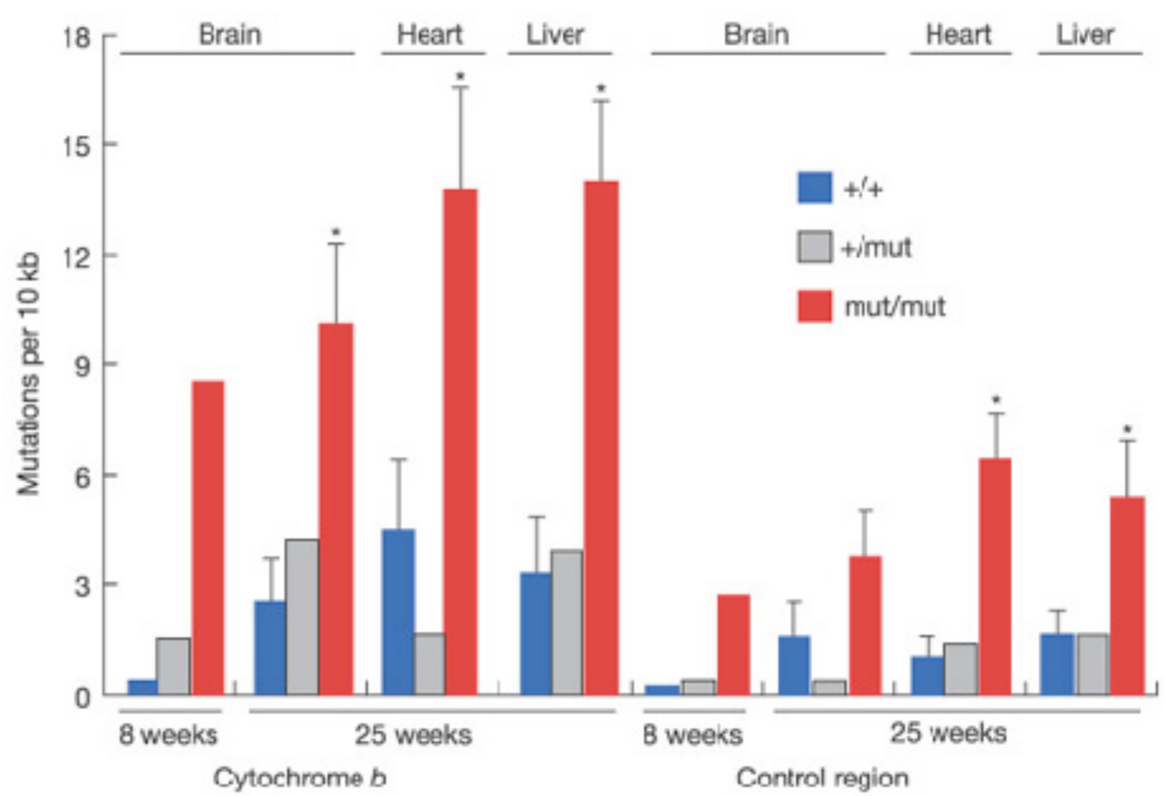
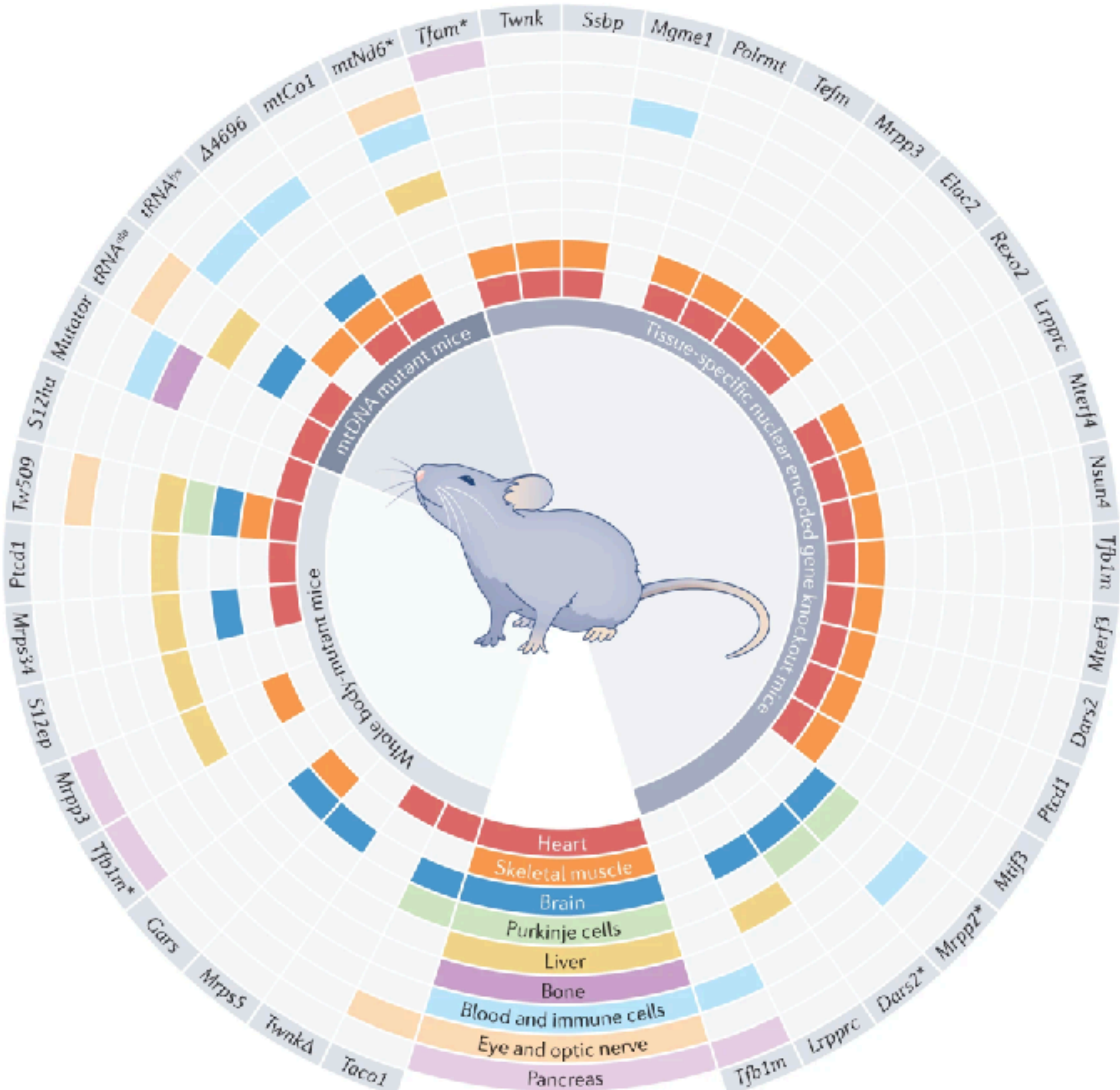
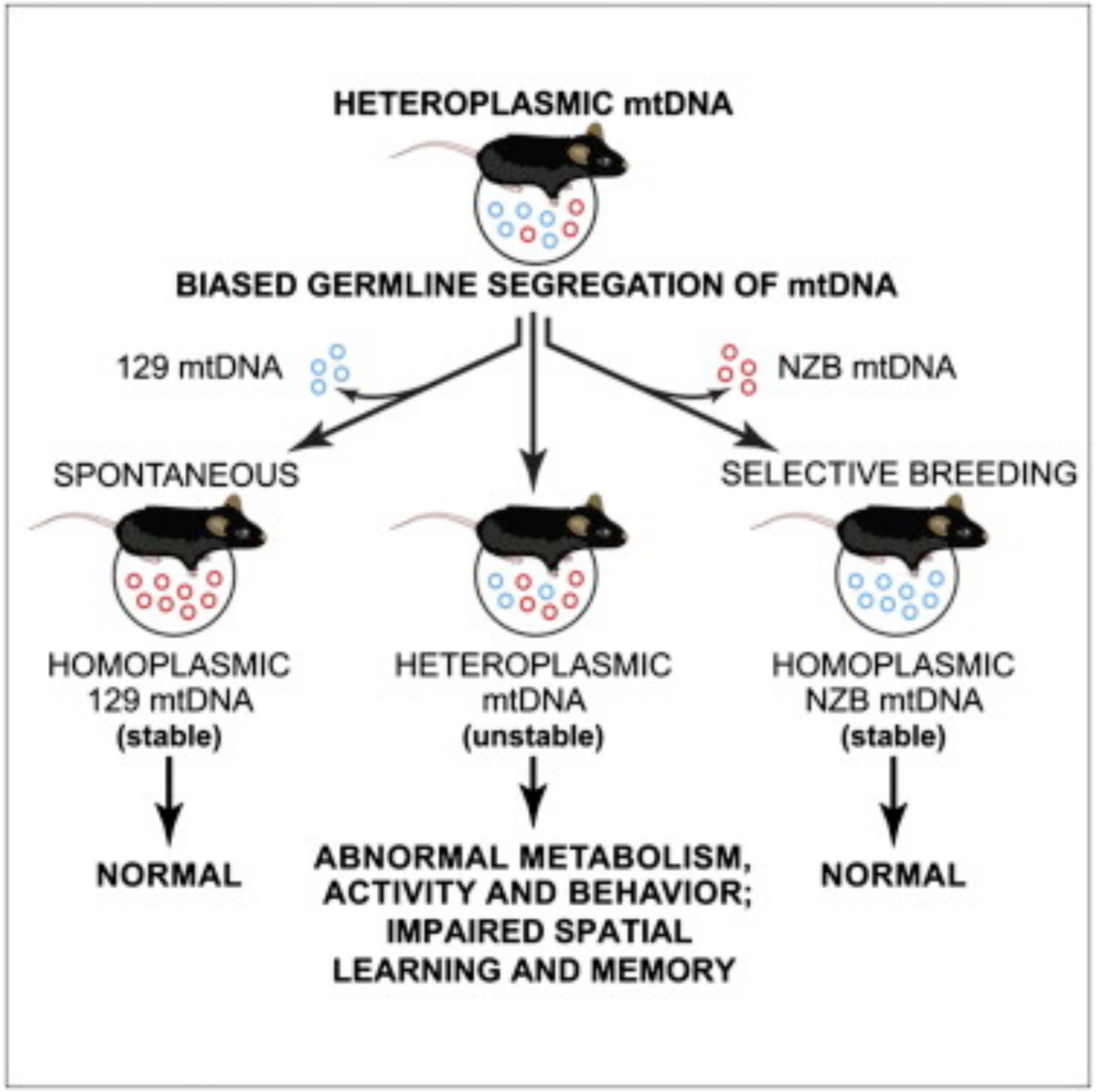


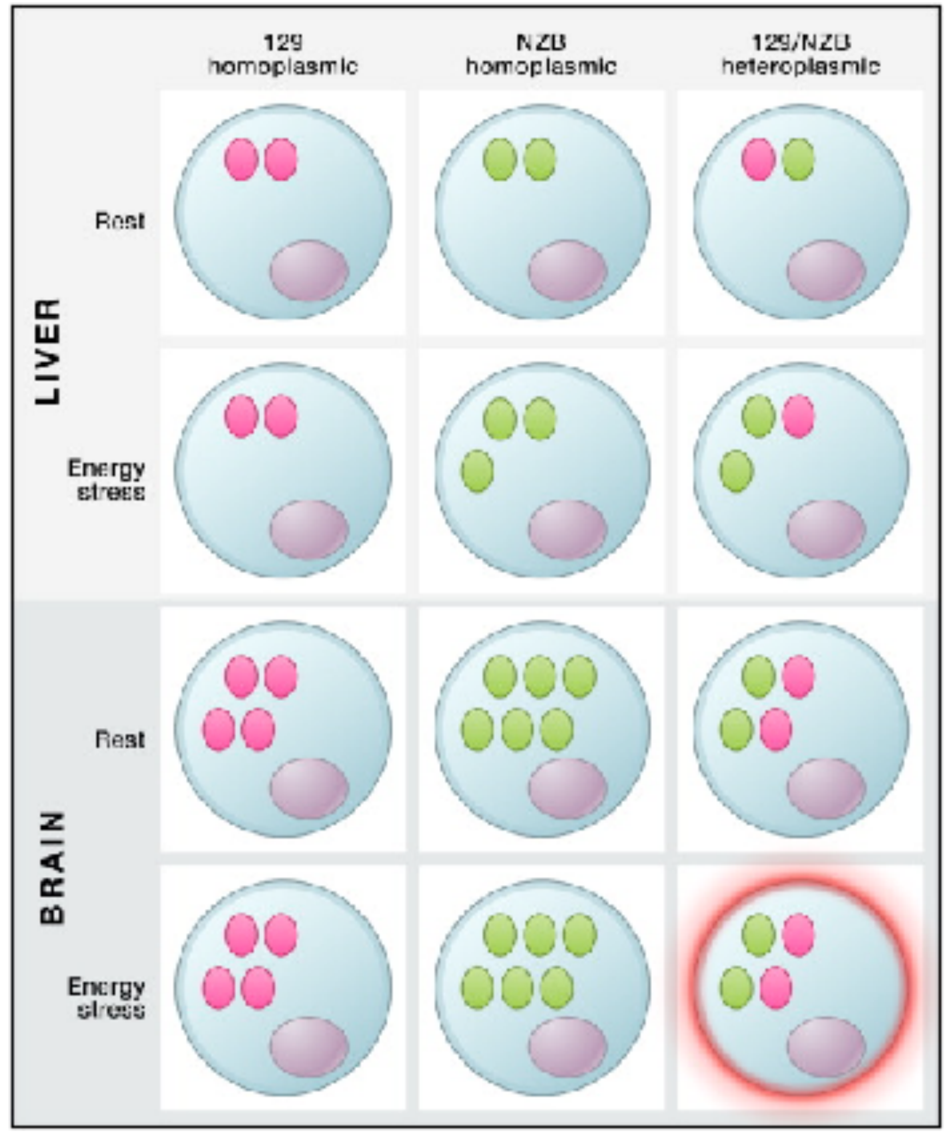
Fig. 6: Genetic models of impaired mitochondrial gene expression.

From: Organization and expression of the mammalian mitochondrial genome



is heteroplasmy bad even in the absence of mutations?





Sharpley et al., 2012

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PLoS BIOLOGY

Strong Purifying Selection in Transmission of Mammalian Mitochondrial DNA

James Bruce Stewart[1*], Christoph Freyer[1], Joanna L. Elson[2], Anna Wredenberg[1], Zekiye Cansu[1], Aleksandra Trifunovic[1], Nils-Göran Larsson[1*]