《医学基因组学进展》

线粒体基因组学

（Mitochondrial genome ）

医学遗传学与发育生物学教研室 梁亮

2017年05月

2015: 3 Parent Babies

May Happen Soon!

2016: 3 Parent Babies

are already here!

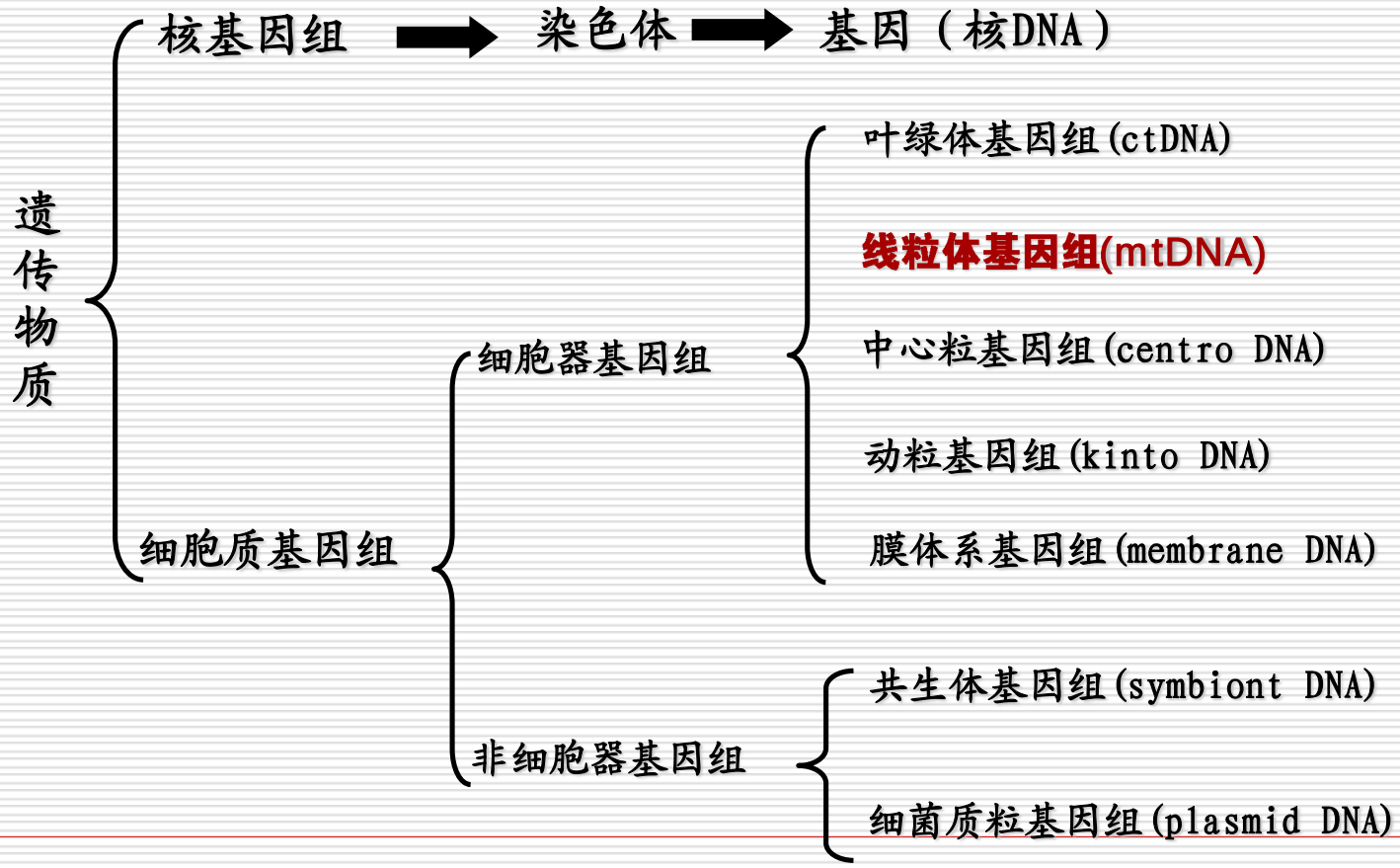
授课内容

一、线粒体基因组

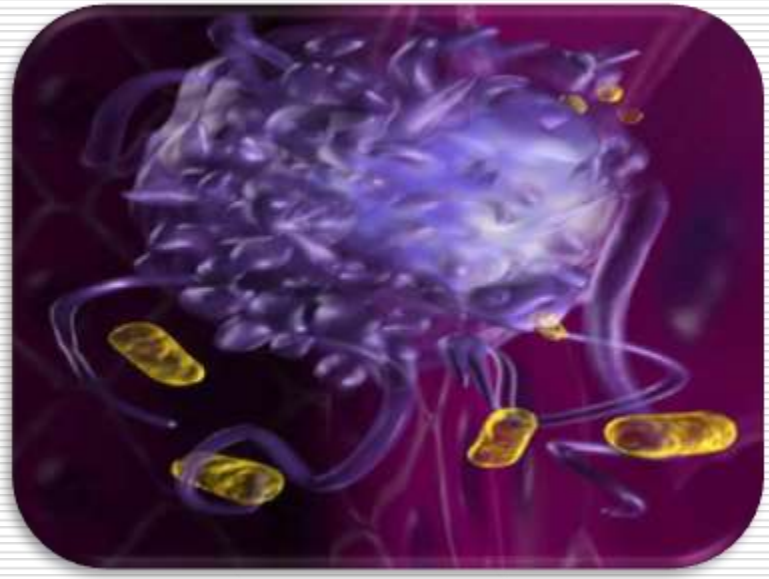
二、线粒体基因组的遗传学基础

三、线粒体基因组与疾病

遗传物质


遗传物质
核基因组
染色体
基因（核DNA）
细胞质基因组
细胞器基因组
叶绿体基因组(ctDNA)
线粒体基因组(mtDNA)
中心粒基因组(centro DNA)
动粒基因组(kinto DNA)
膜体系基因组(membrane DNA)
非细胞器基因组
共生体基因组(symbiont DNA)
细菌质粒基因组(plasmid DNA)

Where do Mitochondria come from?



- **Originally from small bacteria swallowed by eukaryotic cell.**

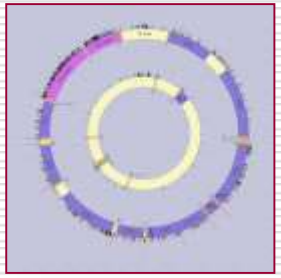
How do we know they evolved from bacteria?

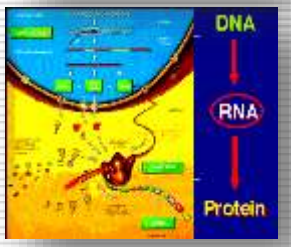
-They have there own circular genome, like bacteria

-Double membrane:
Original + Phagosome

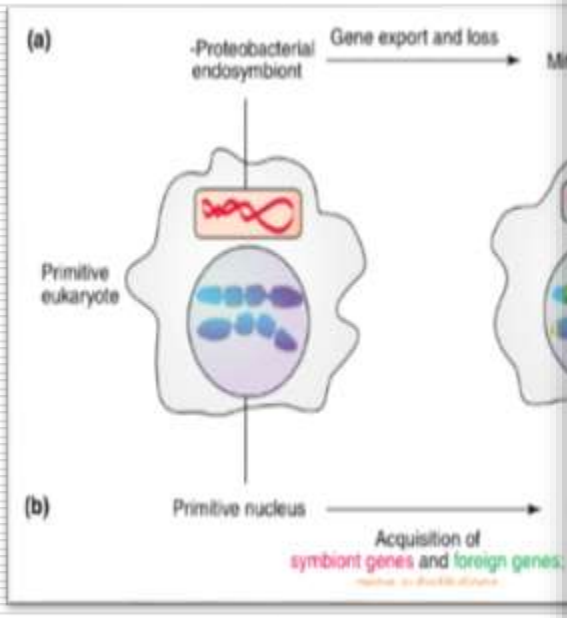
-Protein-synthesis machinery, resembles bacteria

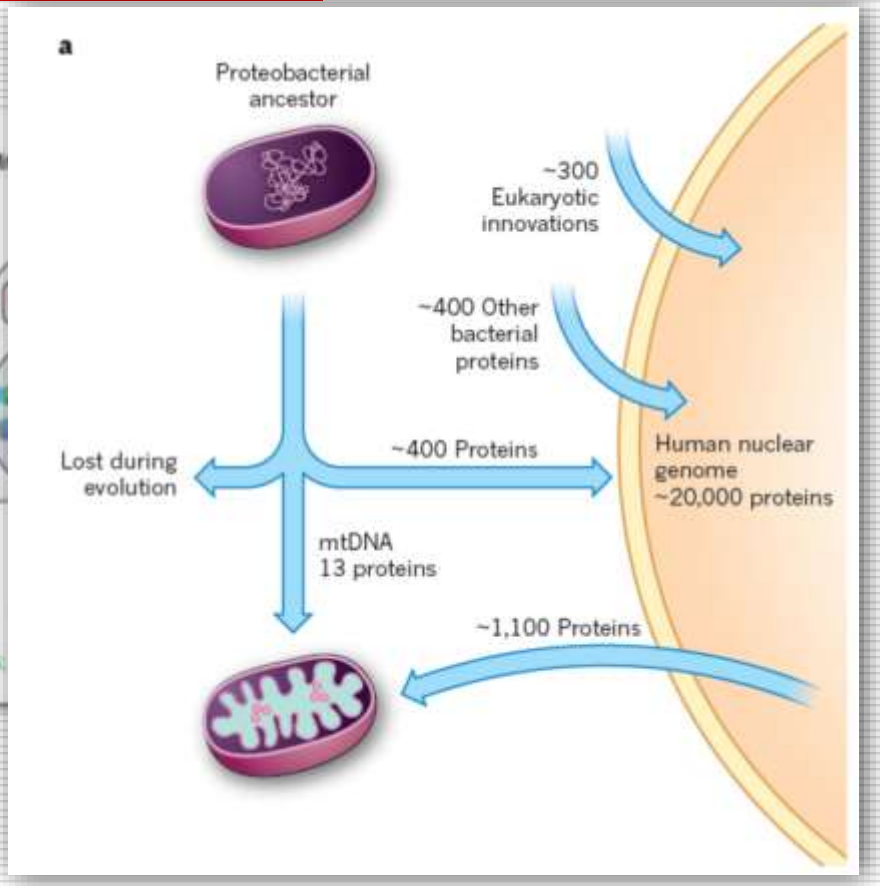
- And more...





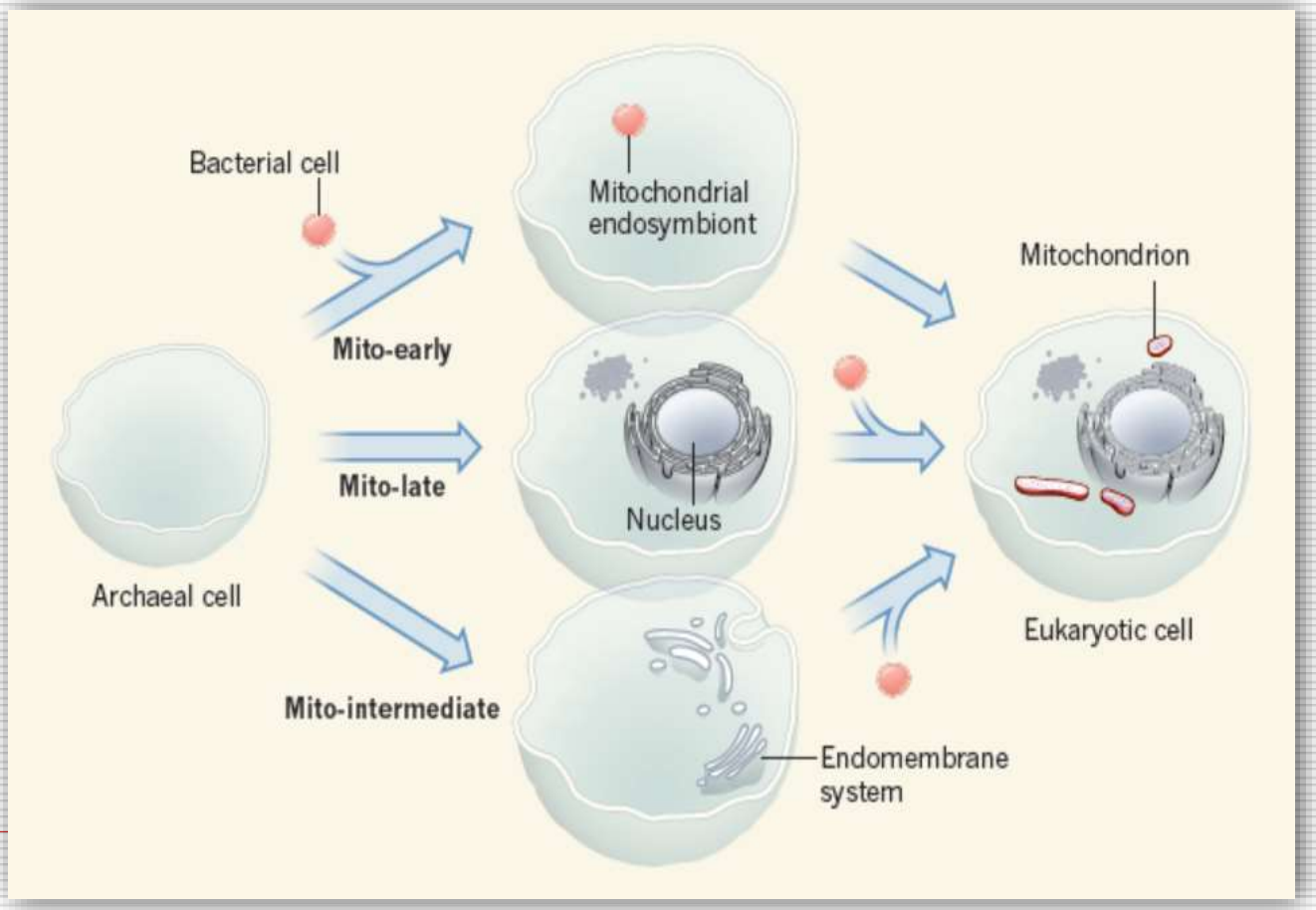
How do we know they evolved from bacteria?



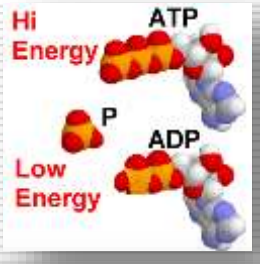


Mitochondria in the second act

Nature. 2016 Mar 3



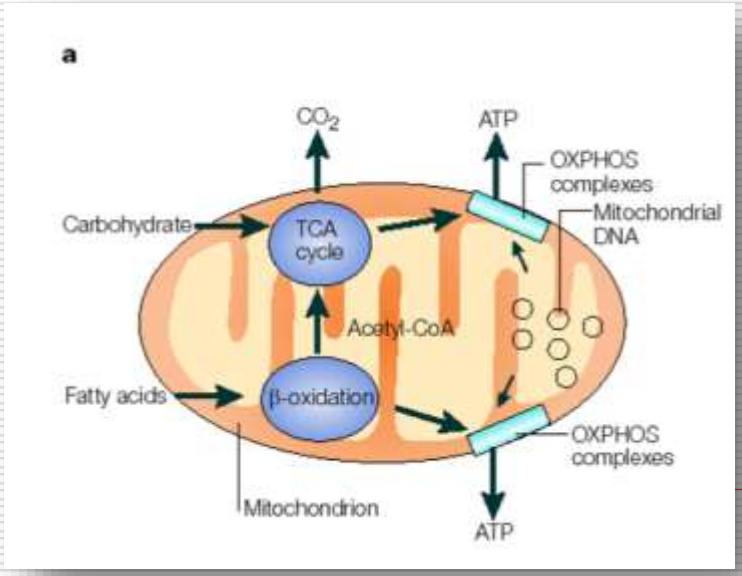
线粒体的功能

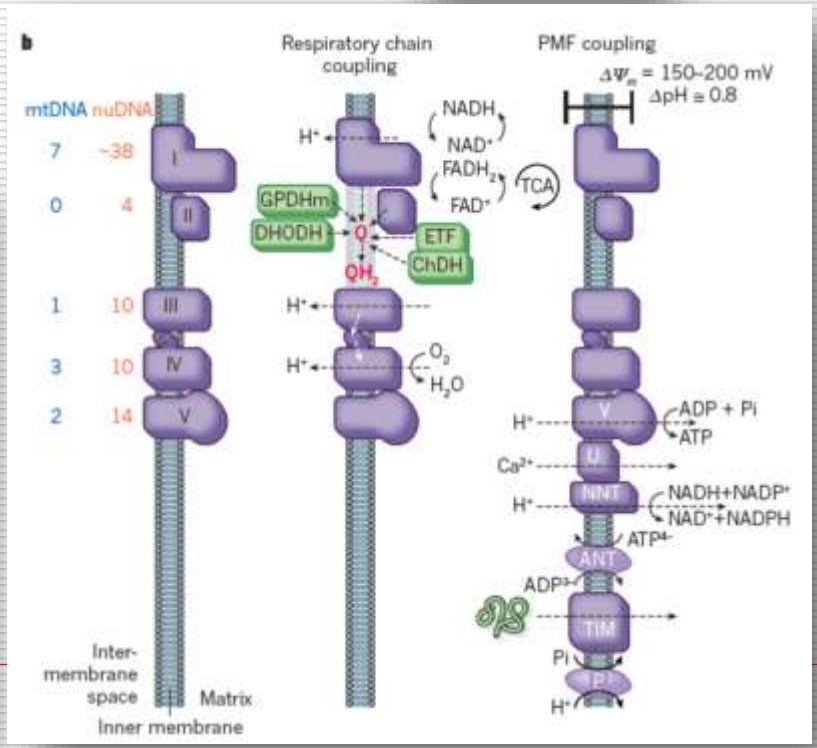


-They are responsible for making ATP, the cell energy-coin

-Krebs cycle

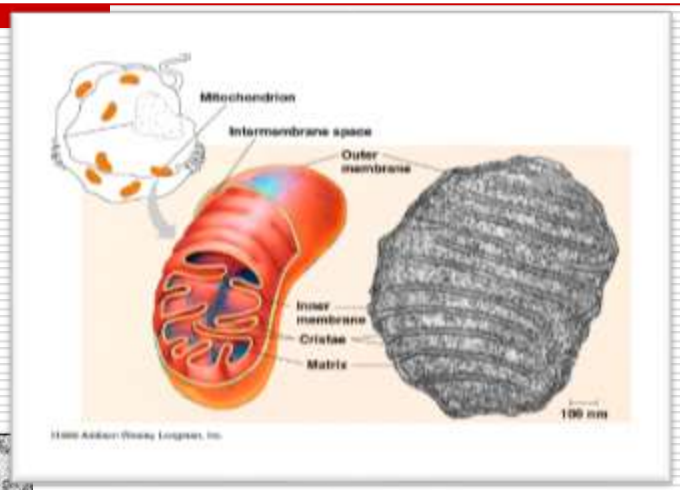
-Electron Transport

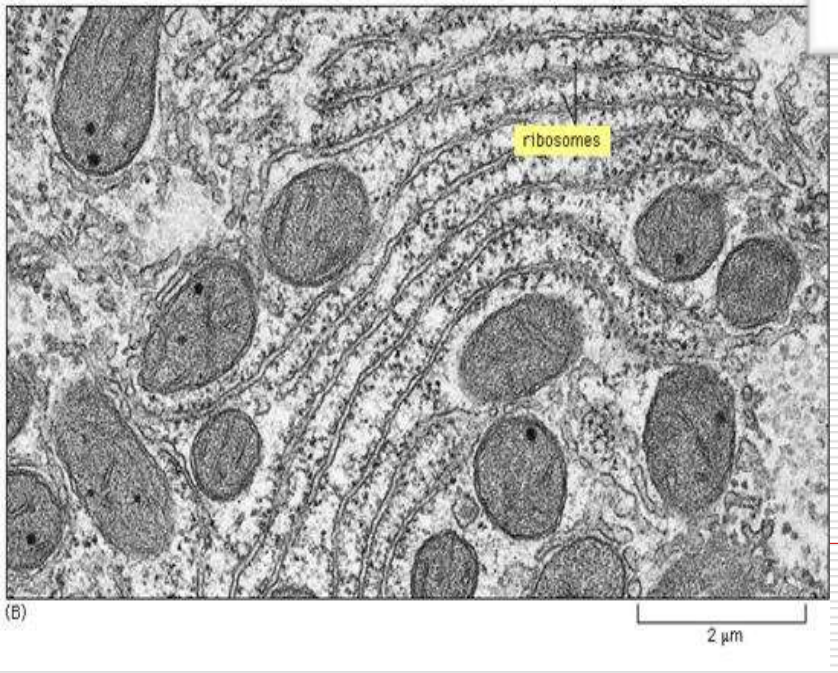




What are Mitochondria?

-Mitochondria are organelles living inside the cell

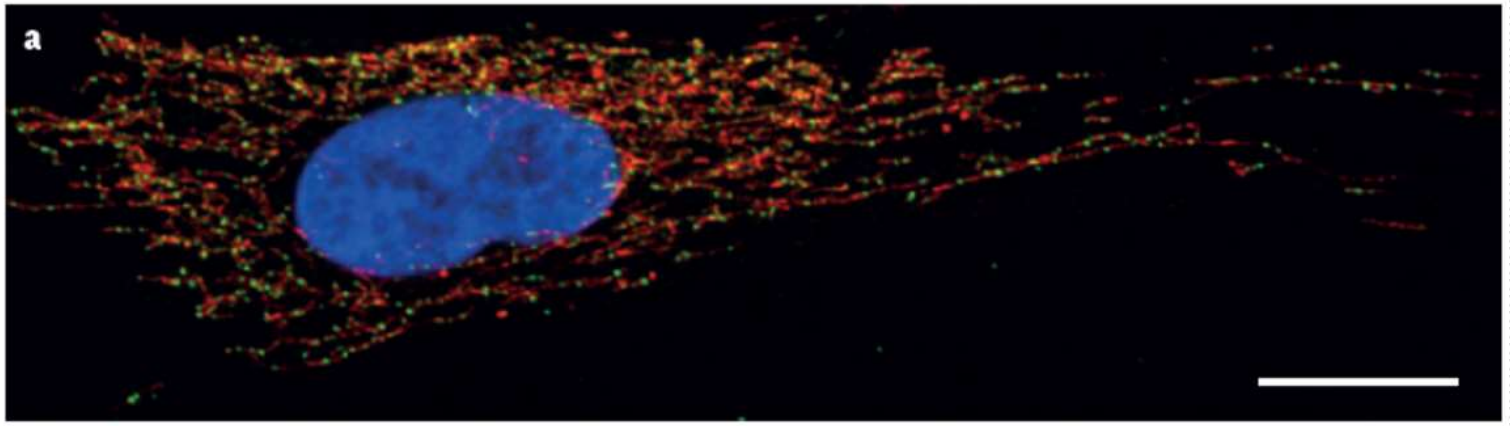




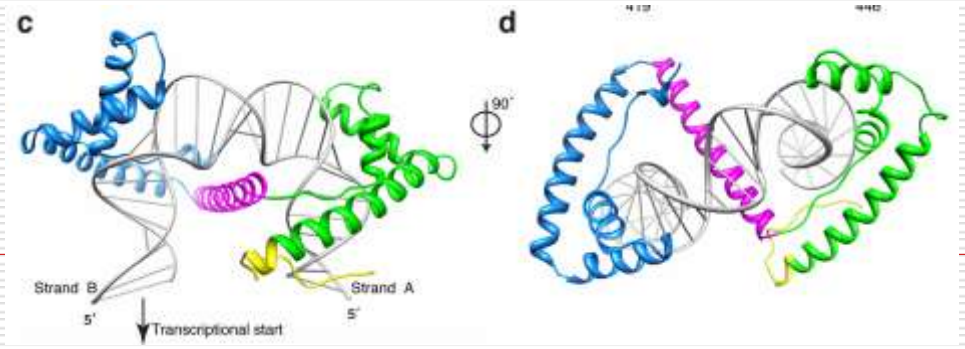
依靠肌动蛋白和微管蛋白运动

线粒体合胞体与拟核

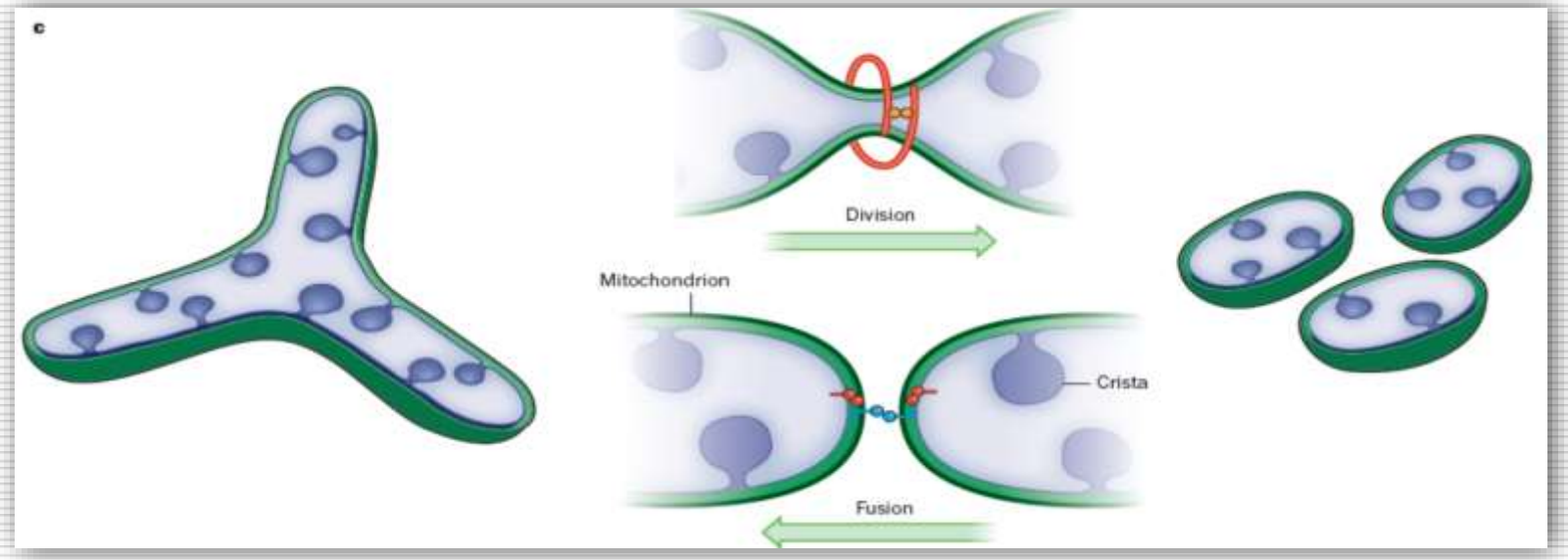
-syncytium & nucleoids



-TFAM(mitochondrial transcription factor A)



线粒体分裂和融合



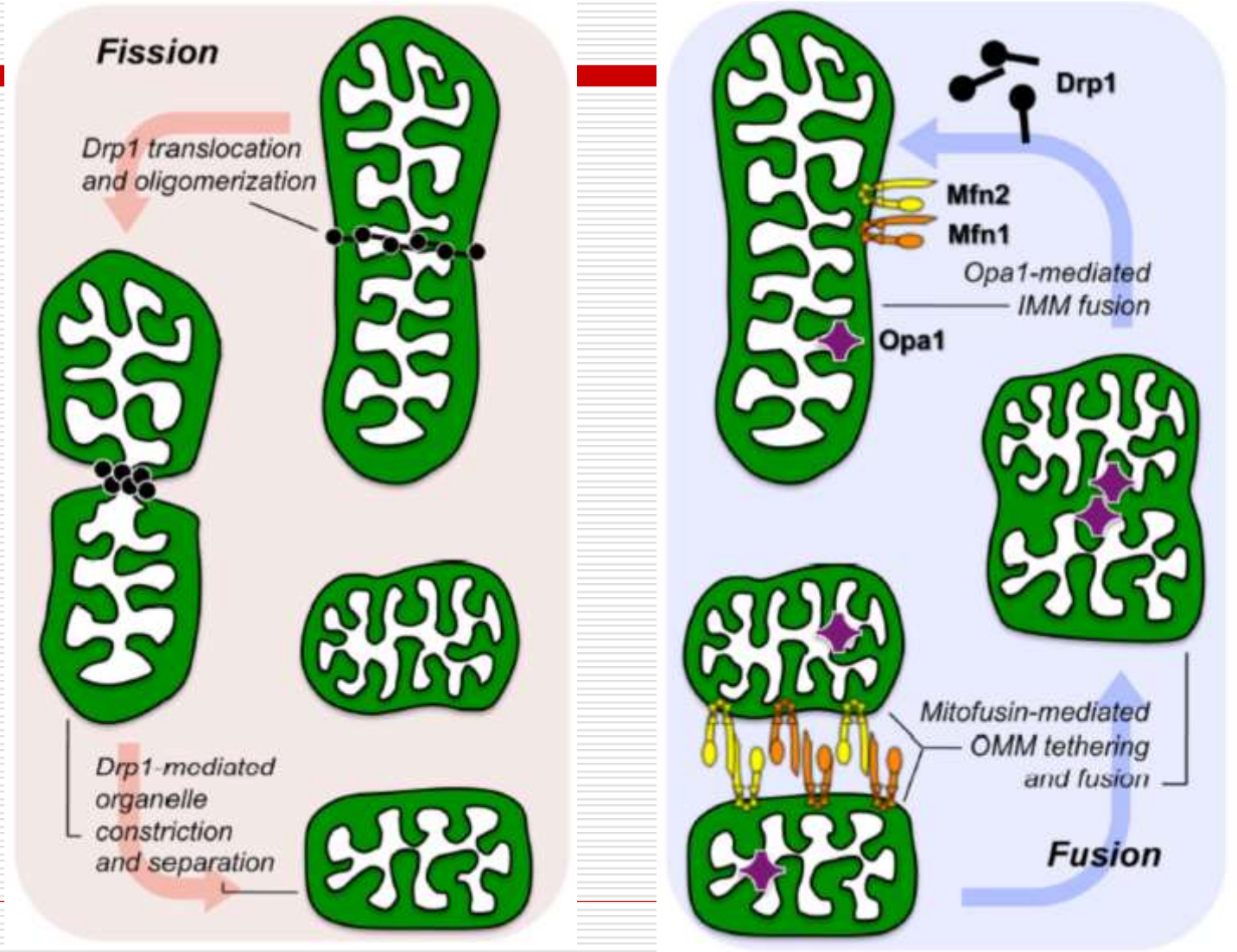
- **与Mt分裂相关的分子：**

  Drp1/Dnm1, Fis1, Fis2, Caf4p, Mdvlp等

- **与Mt融合相关的分子：**

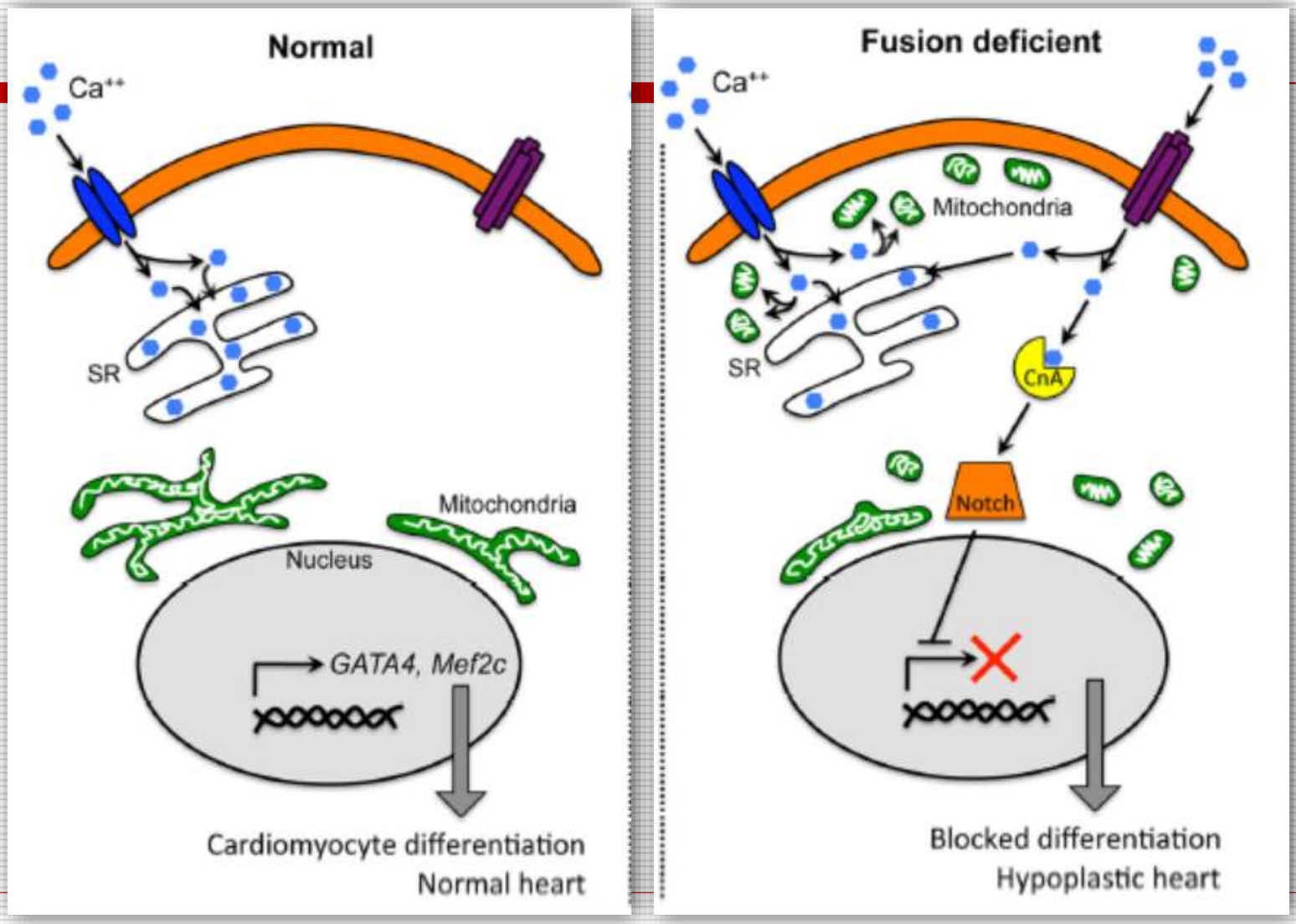
  Fzo1/Mfn1, Mfn2, OPA1, Mgm1等；

线粒体分裂和融合



——*Circ Res*. 2015 January 2; 116(1)

线粒体融合与心脏发育

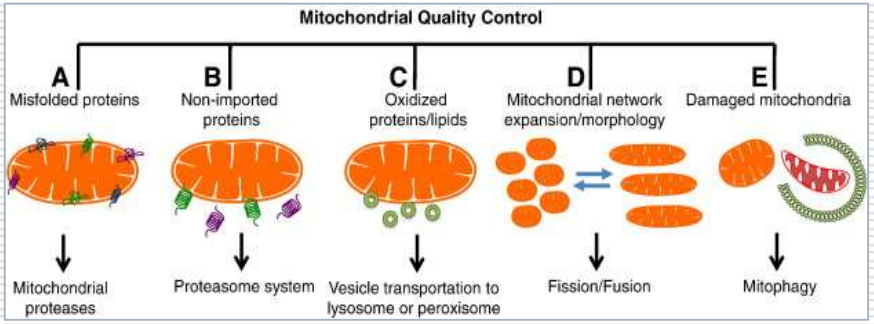


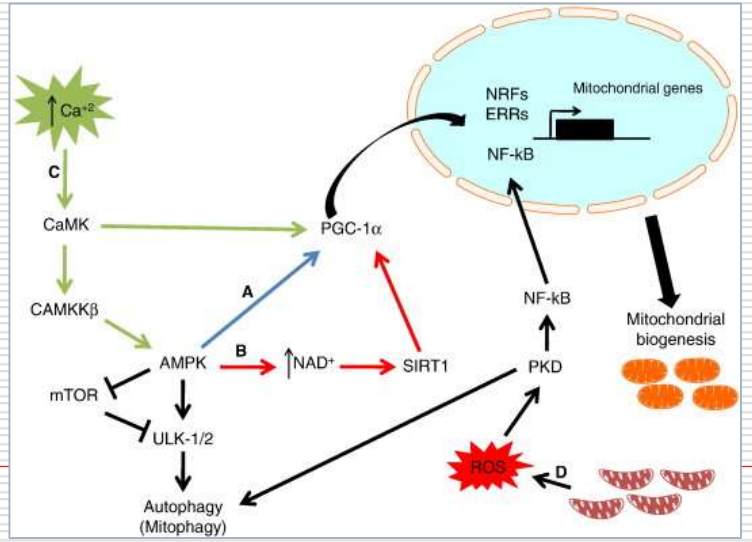
——*Circ Res*. 2015 January 2; 116(1)

线粒体凋亡

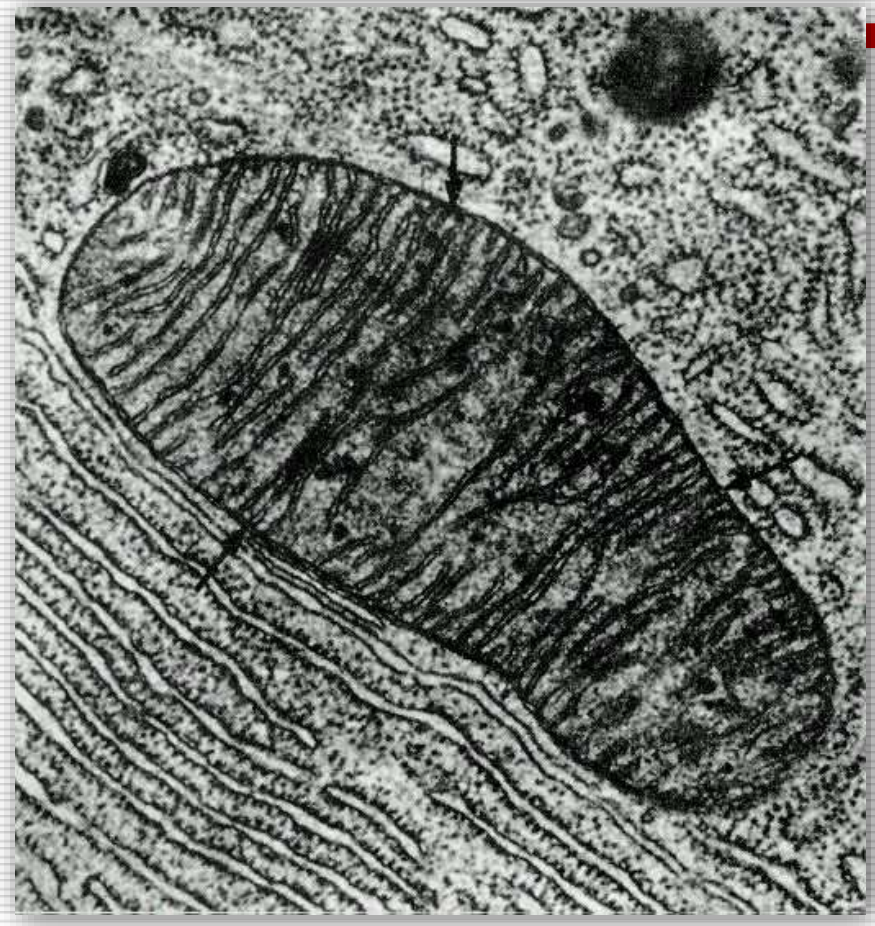
线粒体自噬（Mitochondrial autophagy or mitophagy）

在氧化损伤、营养缺乏、细胞衰老等外界刺激下，细胞内的线粒体发生去极化损伤。损伤线粒体被特异性的包裹进自噬体与溶酶体融合，从而使损伤线粒体降解，维持细胞内环境的稳定。





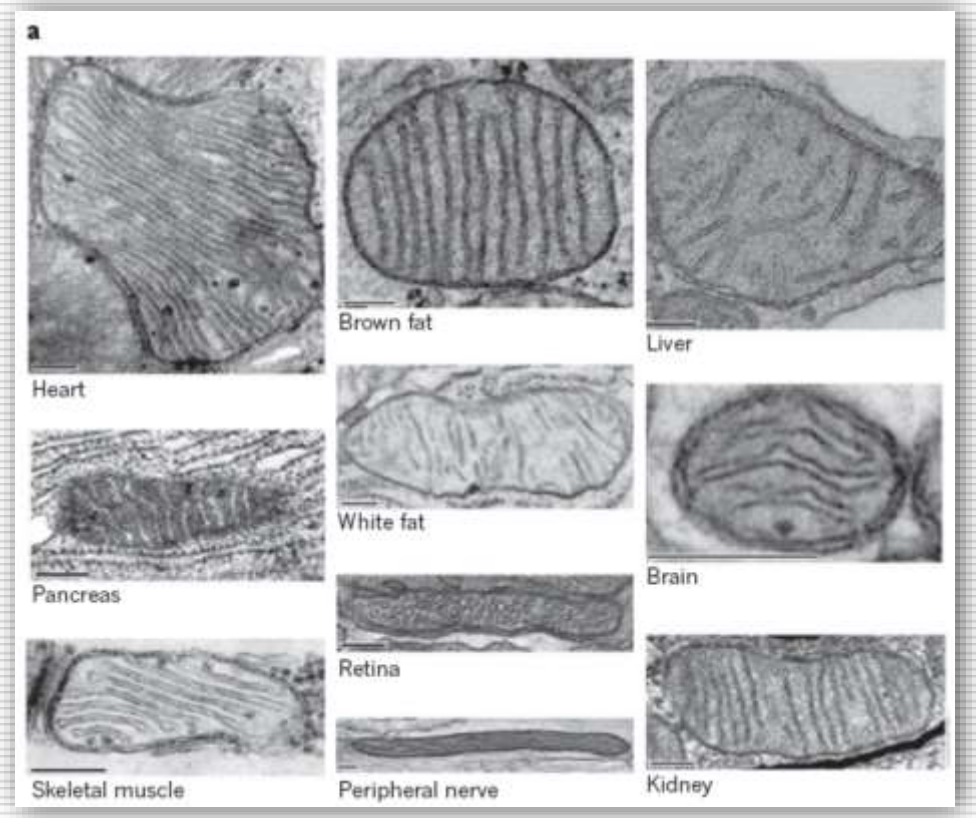
人类线粒体



人类的每一个体细胞平均含有数百个线粒体（除成熟的RBC外）

人类线粒体基因组

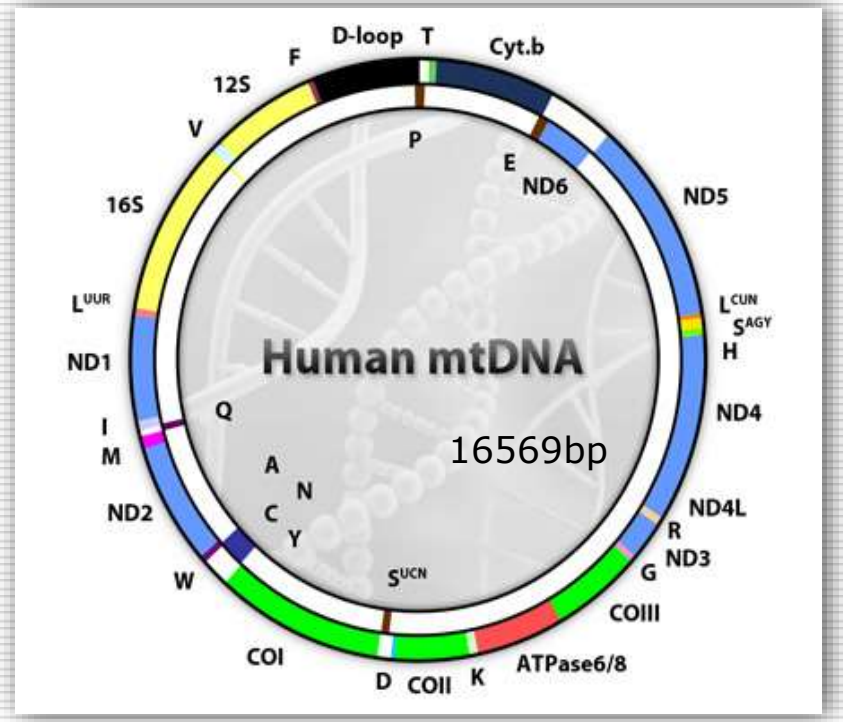
Mitochondrial genome（mtDNA）



每个线粒体内有2～10个拷贝的双链mtDNA，mtDNA占到了细胞总DNA的1%

1963 Nass mtDNA
(独立的遗传体系)

人类线粒体基因组



- 双链16569bp，富含G的称为重链（heavy chain，H），富含C的为轻链（light chain，L）
- 能自主复制，在细胞内具有多拷贝
- 基因内无内含子，排列紧凑
- 编码序列占93%，编码37个基因

- 线粒体基因组的编码产物共有三类
  - 13条与细胞氧化磷酸化有关的多肽链
  - 2个rRNA，分别是16s和12s
  - 22种tRNA

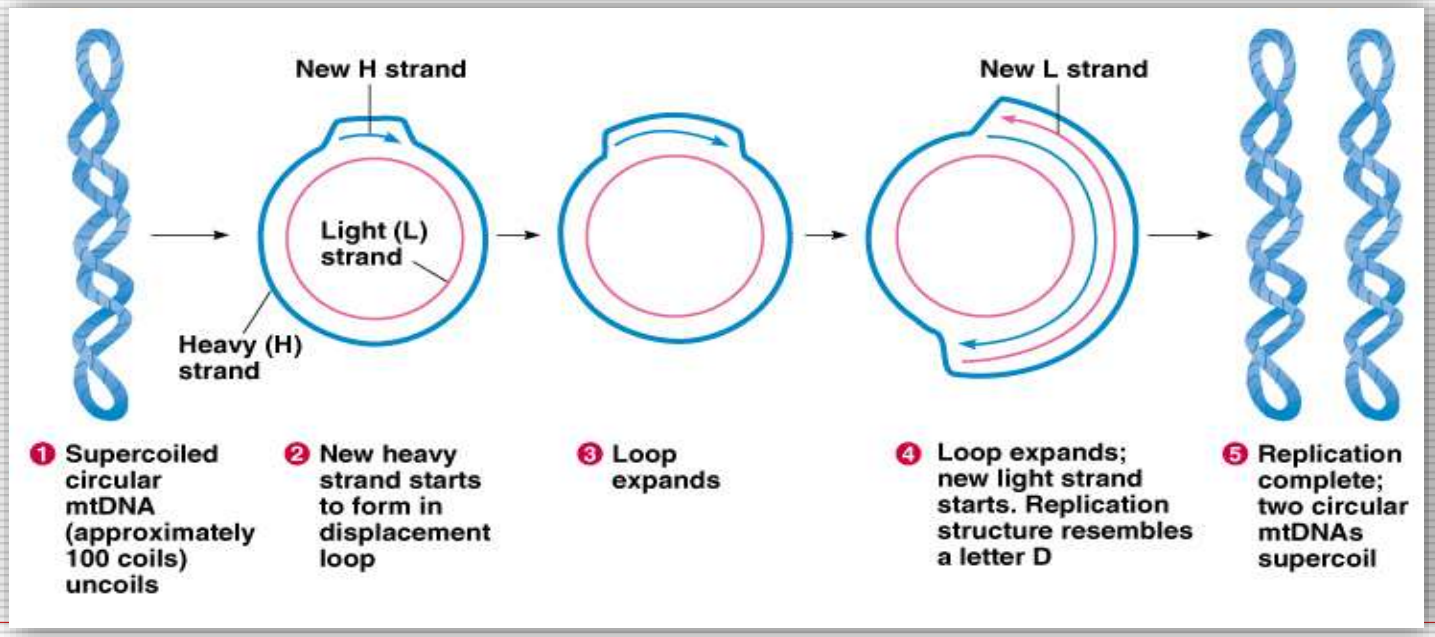
1981 Anderson

“剑桥序列”

线粒体遗传信息的复制、转录和翻译

1、复制方式：半保留复制，由线粒体DNA聚合酶完成

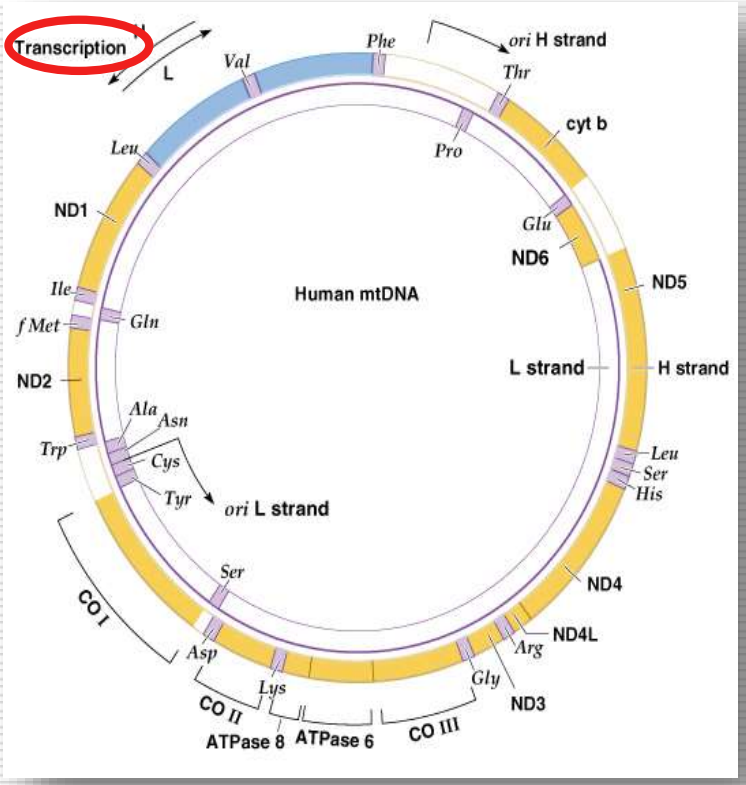
- Replication starts with the H strand.



线粒体遗传信息的复制、转录和翻译

Transcription:

- **Both strands are transcribed.**
- **The D loop contains one promoter for each strand, and the entire strand is transcribed.**
- **The RNA is then cut into individual RNAs for each gene.**
- **Protein-coding genes are given poly-A tails, and rRNA and tRNA**



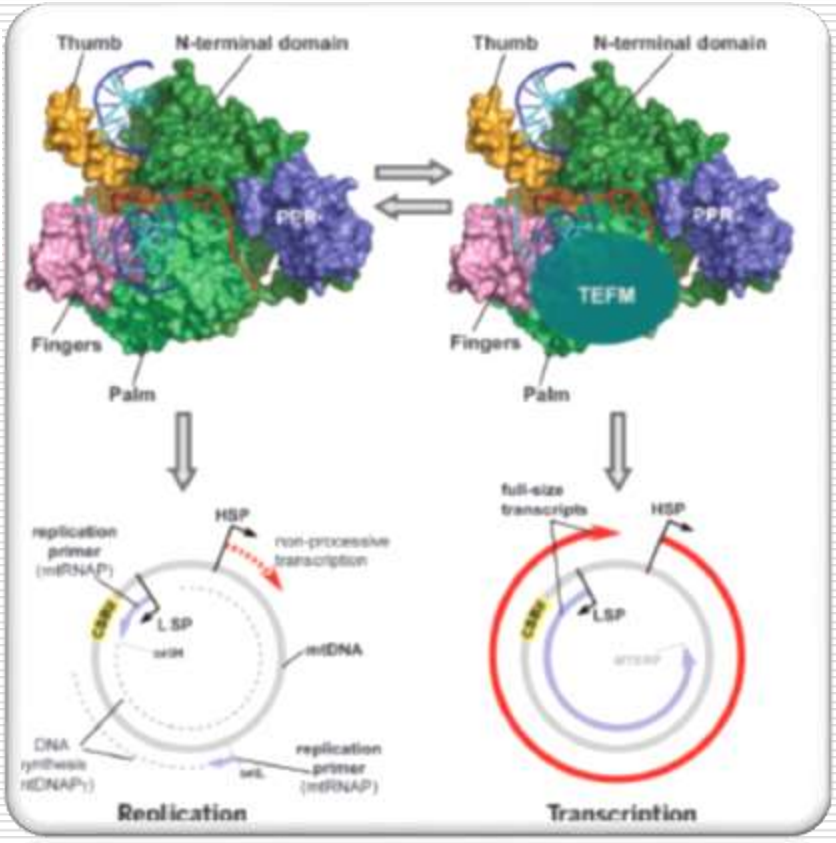
线粒体遗传信息的复制、转录和翻译

MITOCHONDRIAL BIOLOGY

Replication-transcription switch in human mitochondria

Karen Agaronyan, Yaroslav I. Morozov, Michael Anikin, Dmitry Temiakov*

TEFM——
线粒体转录延伸因子



Temiakov et al., Science,2015

线粒体遗传信息的复制、转录和翻译

2. **有自身的核糖体，且不同的生物差异大：**

人的Hela细胞线粒体核糖体为60S，由45S和35S两个亚基组成，而细胞质核糖体为74S；

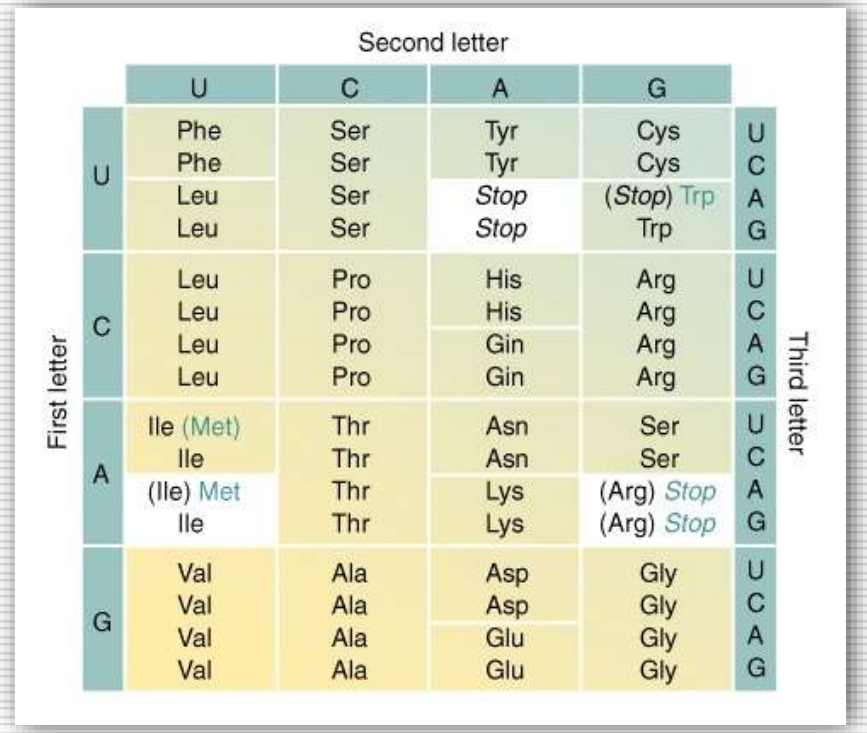
酵母核糖体（75S）和各种RNA（rRNA、tRNA和mRNA），而细胞质核糖体为80S。

3. **线粒体的密码子与核基因密码子有差异：最为显著的是**

mtDNA中UGA编码色氨酸，而非终止信号；tRNA兼用性较强，仅用22个tRNA来识别多达48个密码子

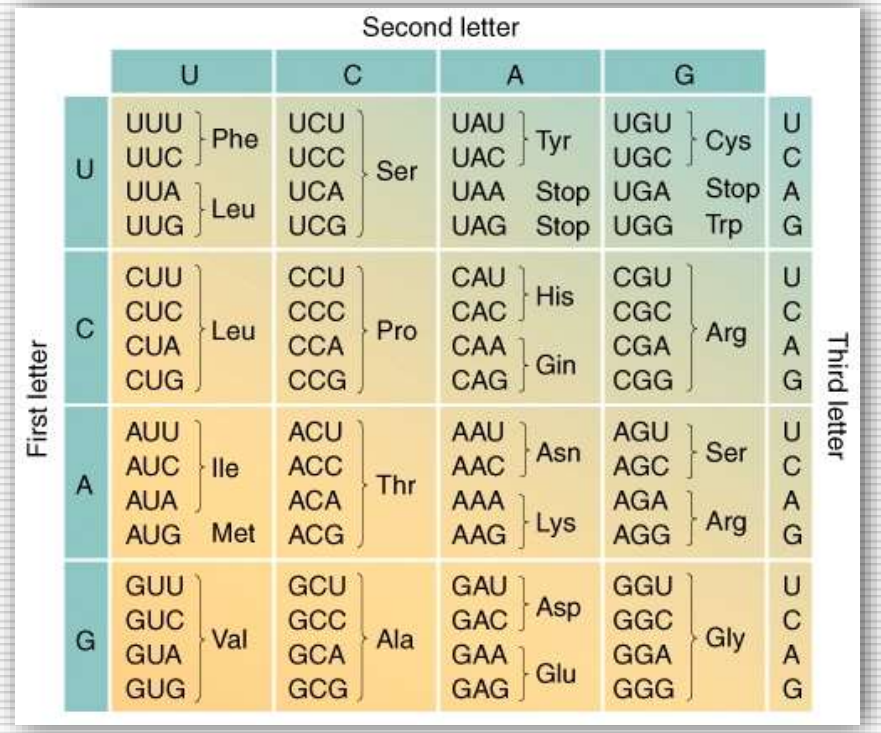
线粒体遗传信息的复制、转录和翻译

Mitochondrial code



First letter	Second letter U	Second letter C	Second letter A	Second letter G	Third letter
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*Stop*	(*Stop*) Trp	A
	Leu	Ser	*Stop*	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gin	Arg	A
	Leu	Pro	Gin	Arg	G
A	Ile (Met)	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	(Ile) Met	Thr	Lys	(Arg) *Stop*	A
	Ile	Thr	Lys	(Arg) *Stop*	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Universal code



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

授课内容

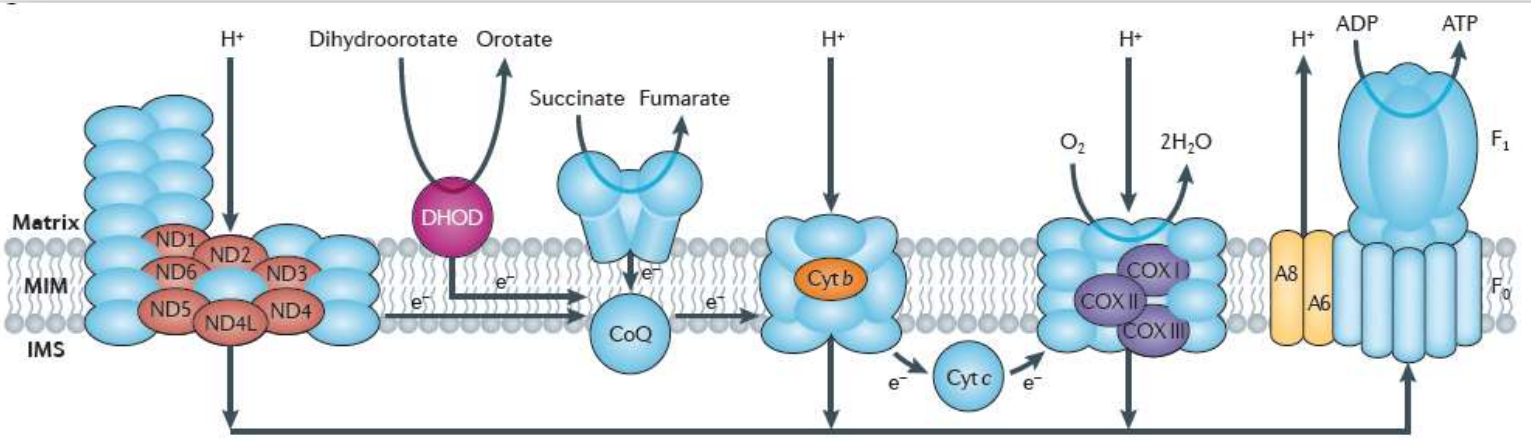
一、线粒体基因组

二、线粒体基因组的遗传学基础

三、线粒体基因组与疾病

mtDNA的遗传特点

1.mtDNA具有半自主性：

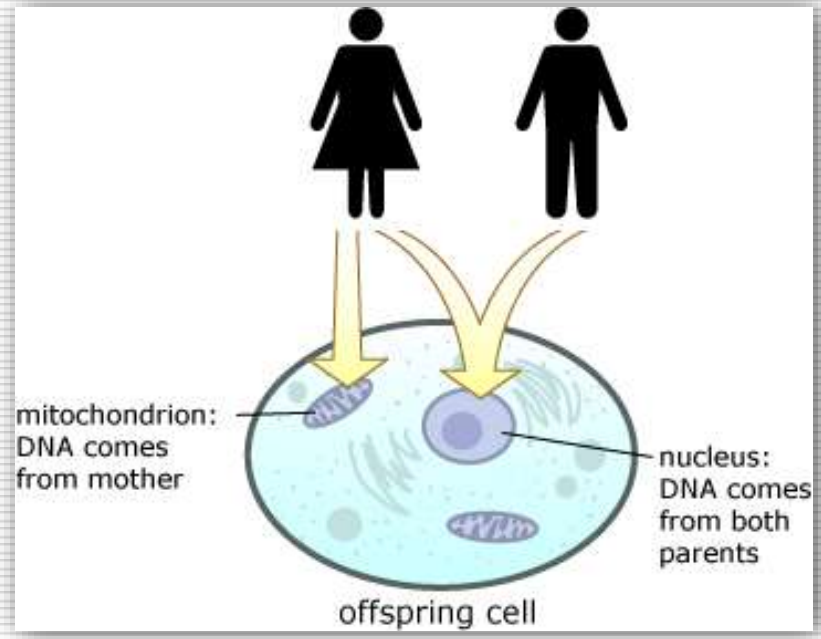


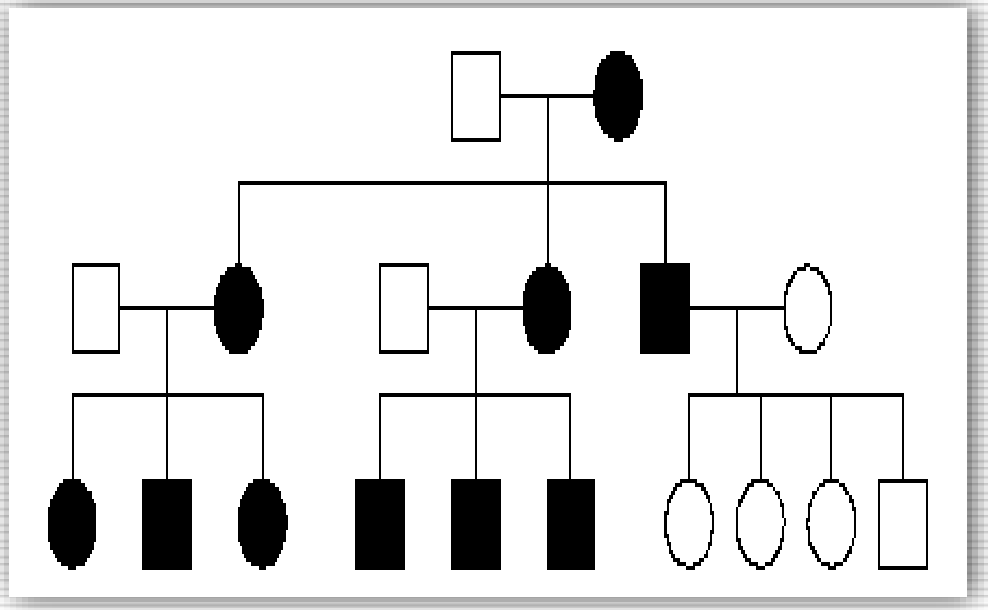
Polypeptides	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded subunits	7	0	1	3	2
nDNA-encoded subunits	~39	4	10	10	~14
Assembly proteins	~11	~2	~9	~30	~3

Schon, DiMauro and Hirano 2012 Nat Rev Genet

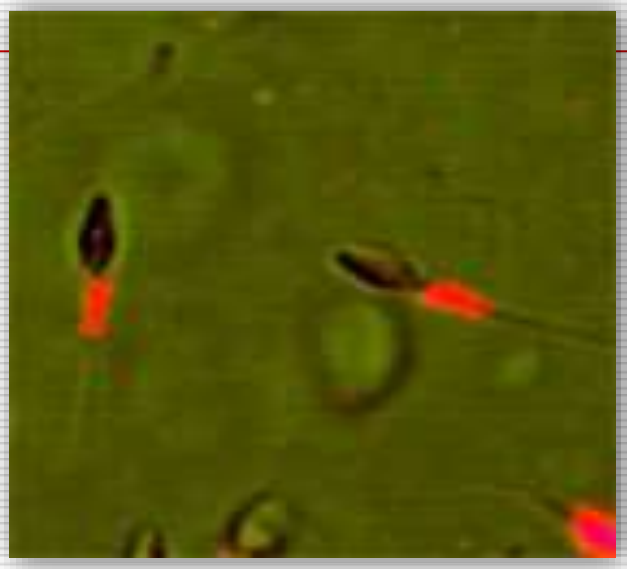
mtDNA的遗传特点

2.mtDNA为**母系**遗传：





mtDNA的遗传特点



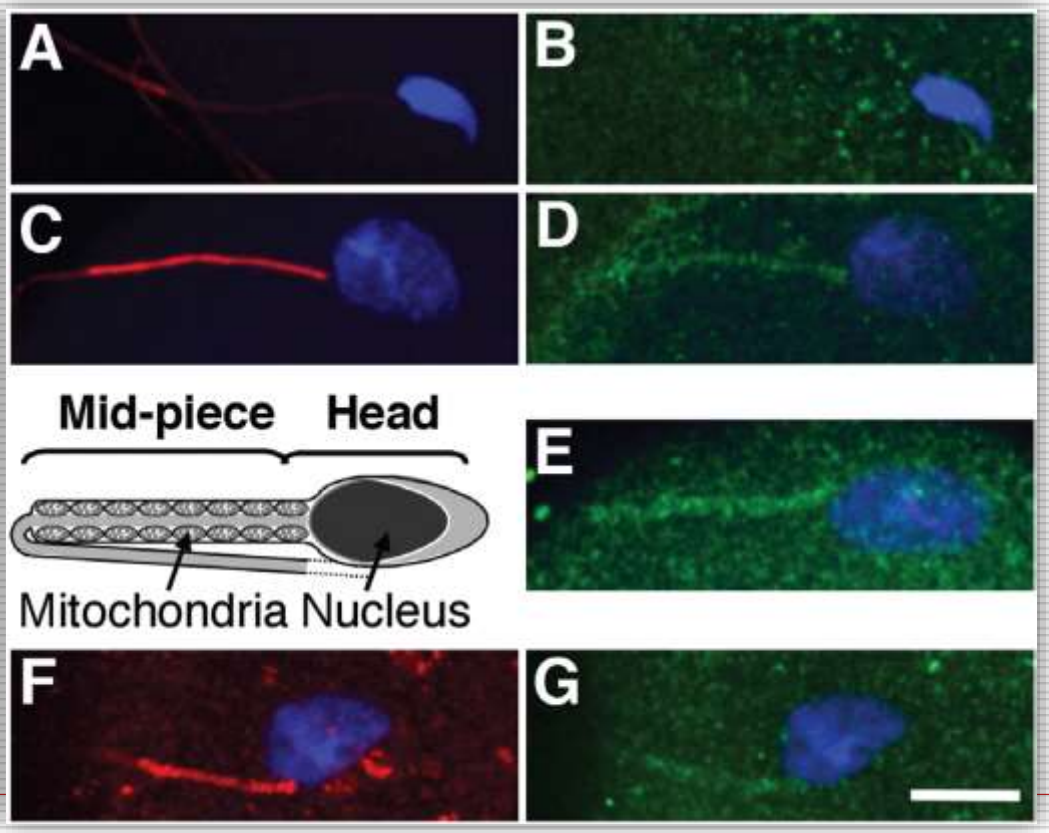
2.mtDNA为母系遗传：

Reason:

- The sperm carries most of its mitochondria its tail and has only about 100 mitochondria compared to 100,000 in the oocyte.
- Although sperm mitochondria penetrate the egg, most are degraded after a few hours.
- As the cells develop, more and more of the mtdna from males is diluted out.
- Hence less than one part in 10^4 or 0.01% of the mtdna is paternal

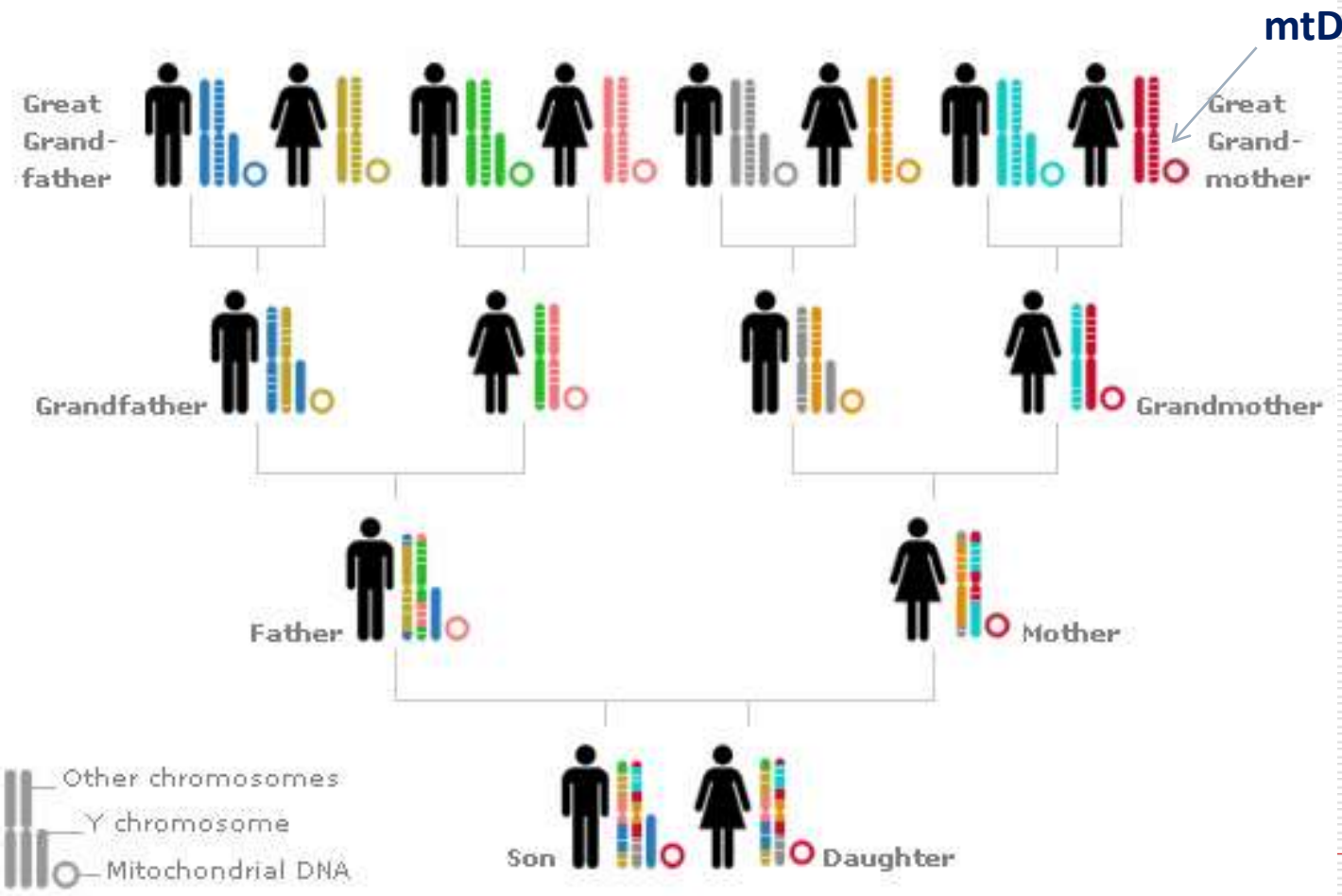
mtDNA的遗传特点

2.mtDNA为**母系**遗传：



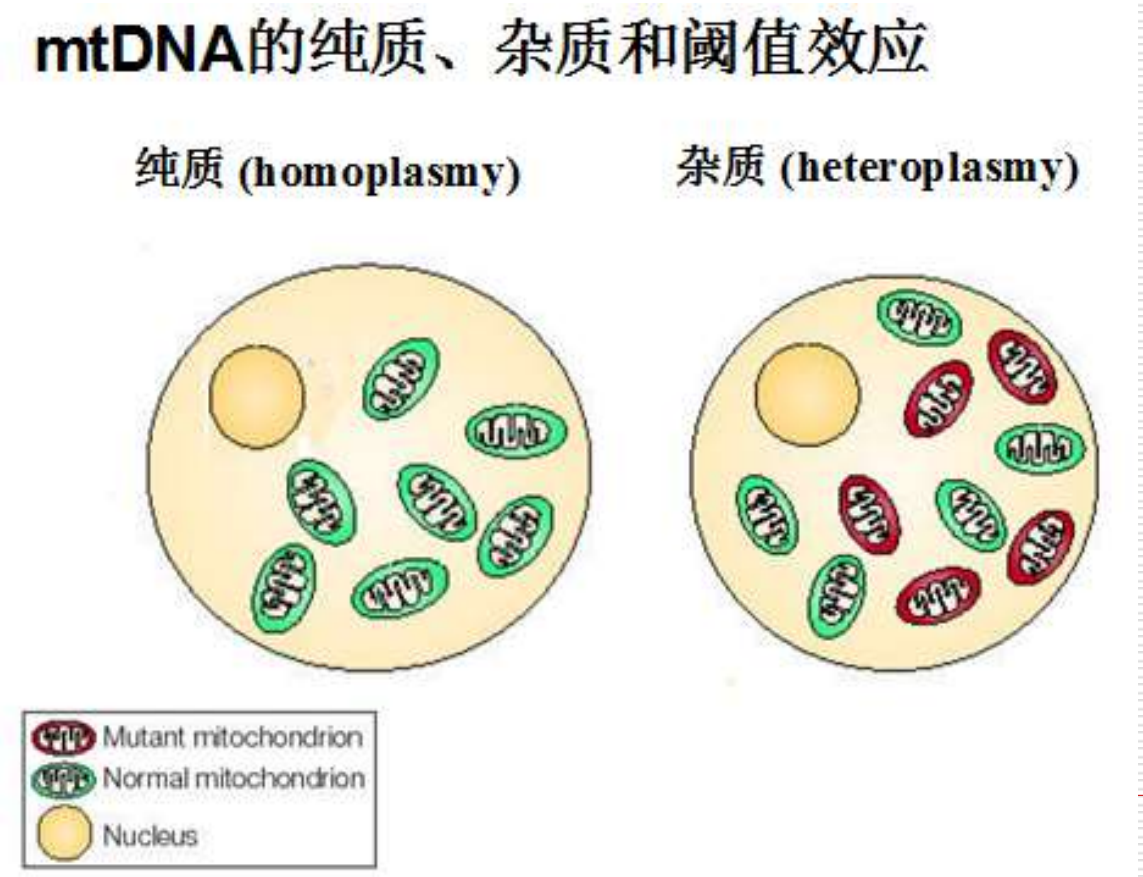
Vincent Galy, Science, 2012

Therefore, the mitochondria that we have are passed down from mother to children



mtDNA的遗传特点

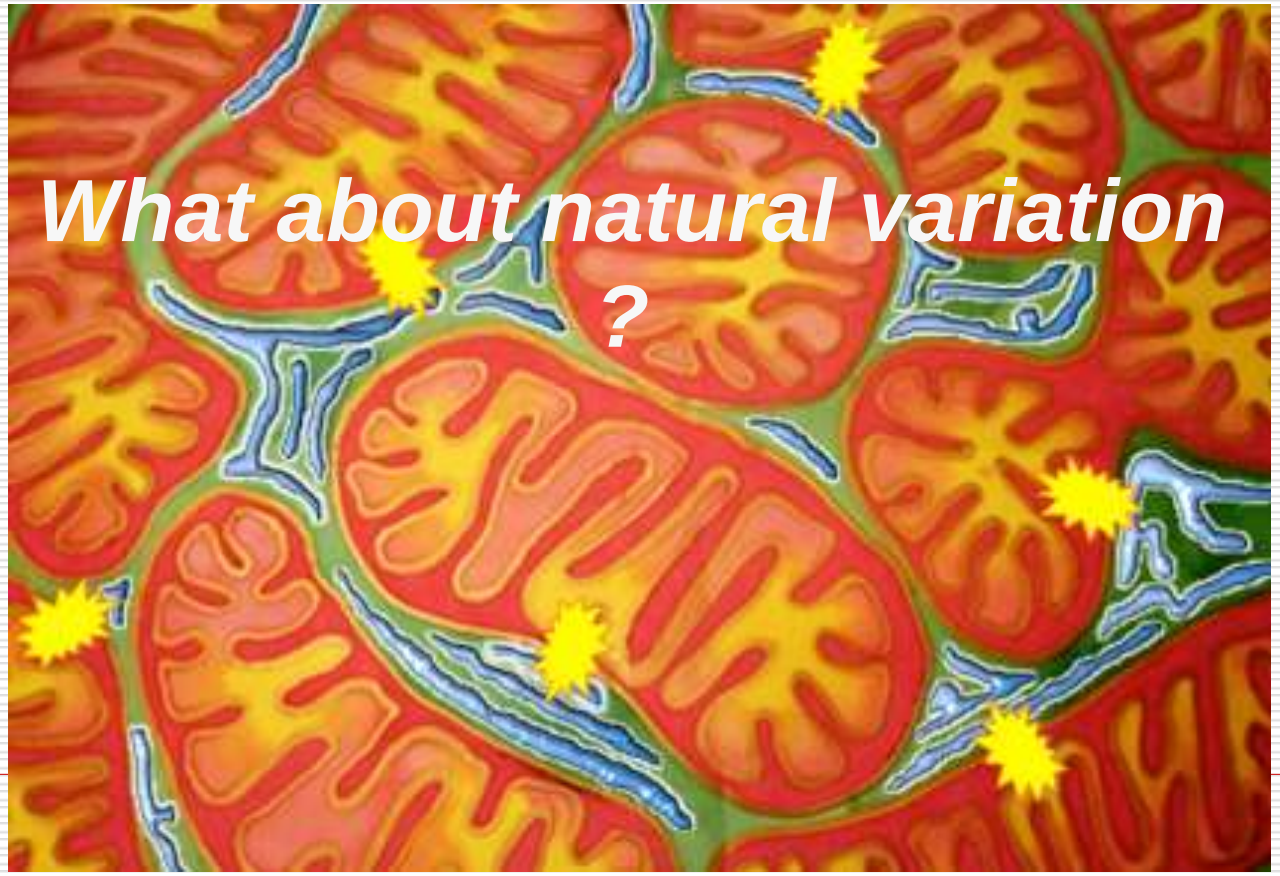
3.mtDNA具阈值效应的特性



mtDNA的遗传特点

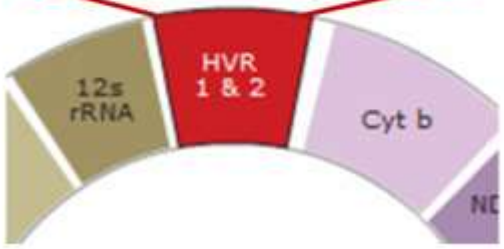
4.mtDNA突变率很高：mtDNA的突变率比核DNA高

10 –



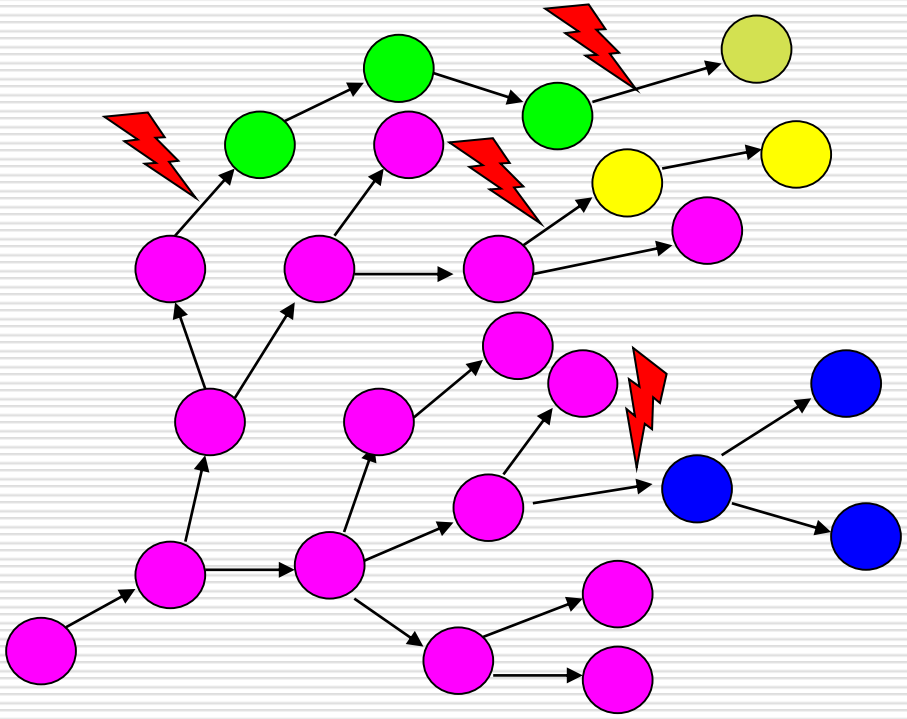
Mitochondrial D-loop (variable region)

ATTCTAATTT AAACTATTCT CTGTTCTTTC ATGGGGAAGC AGATTTGGGT
ACCACCCAAG TATTGACTCA CCCATCAACA ACCGCTATGT ATTTCGTACA
TTACTGCCAG CCACCATGAA TATTGTACGG TACCATAAAT ACTTGACCAC
CTGTAGTACA TAAAAACCCA ATCCACATCA AAACCCCCTC CCCATGCTTA
CAAGCAAGTA CAGCAATCAA CCCTCAACTA TCACACATCA ACTGCAACTC
CAAAGCCACC CCTCACCCAC TAGGATACCA ACAAACCTAC CCACCCTTAA
CAGTACATAG TACATAAAGC CATTTACCGT ACATAGCACA TTACAGTCAA
ATCCCTTCTC GTCCCCATGG ATGACCCCCC TCAGATAGGG GTCCCTTGAC



- D-loop has high mutation rate

"Molecular clock"



Mutation rate:
1 per 3,000 years
(for mitochondrial variable region)

Example

Gene sequence A:
AGACGCCTATATA

Gene sequence B:
AGGCGCCTATATA

Gene sequence C:
AGACGCCTATTTA

Example

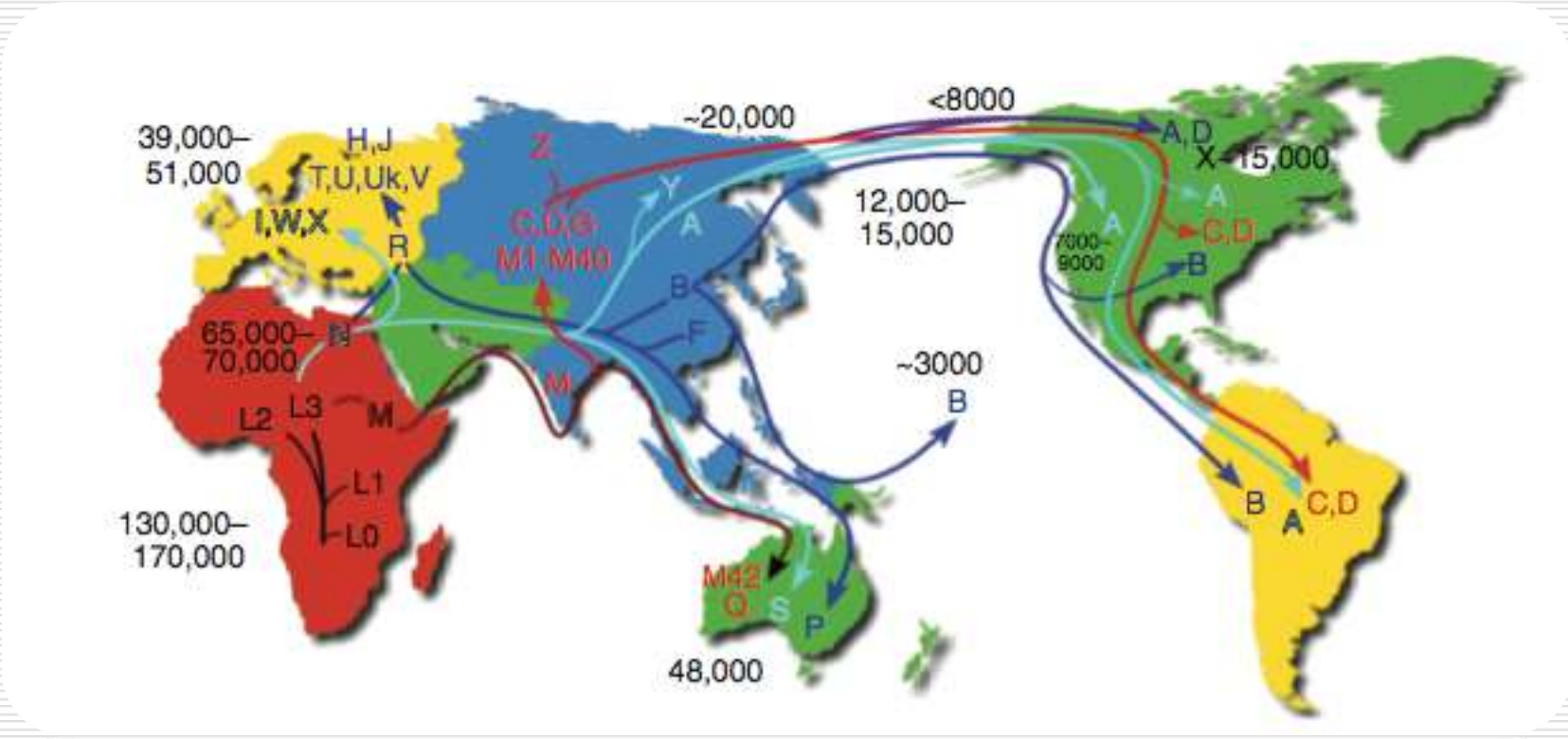
Sequence B is approximately 3,000 years older then sequence A and 6,000 years older then sequence C.

We can determine common ancestral mothers by changes in the sequence of the mitochondrial genome

African Eve or the Mitochondrial Eve

Many researchers take the mitochondrial evidence as support for the "single-origin" or Out-of-Africa model

The migration of maternal clans



线粒体分子进化实验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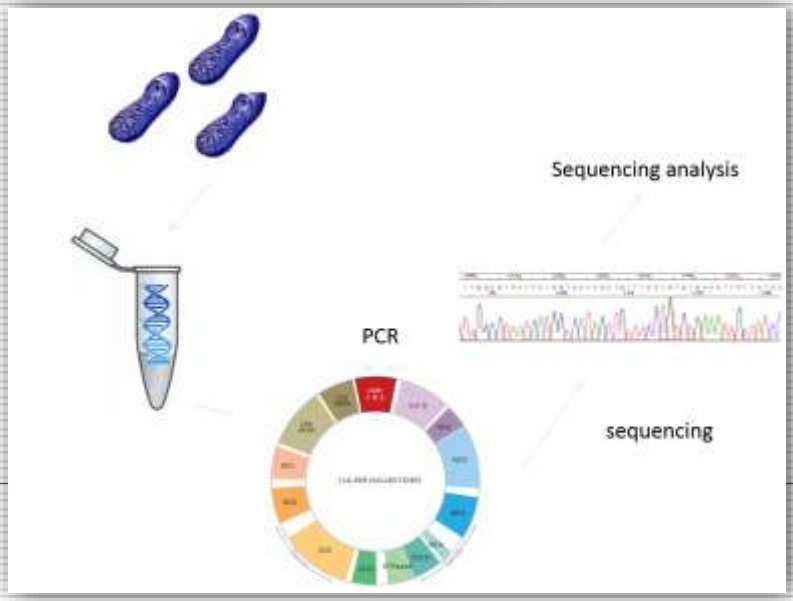
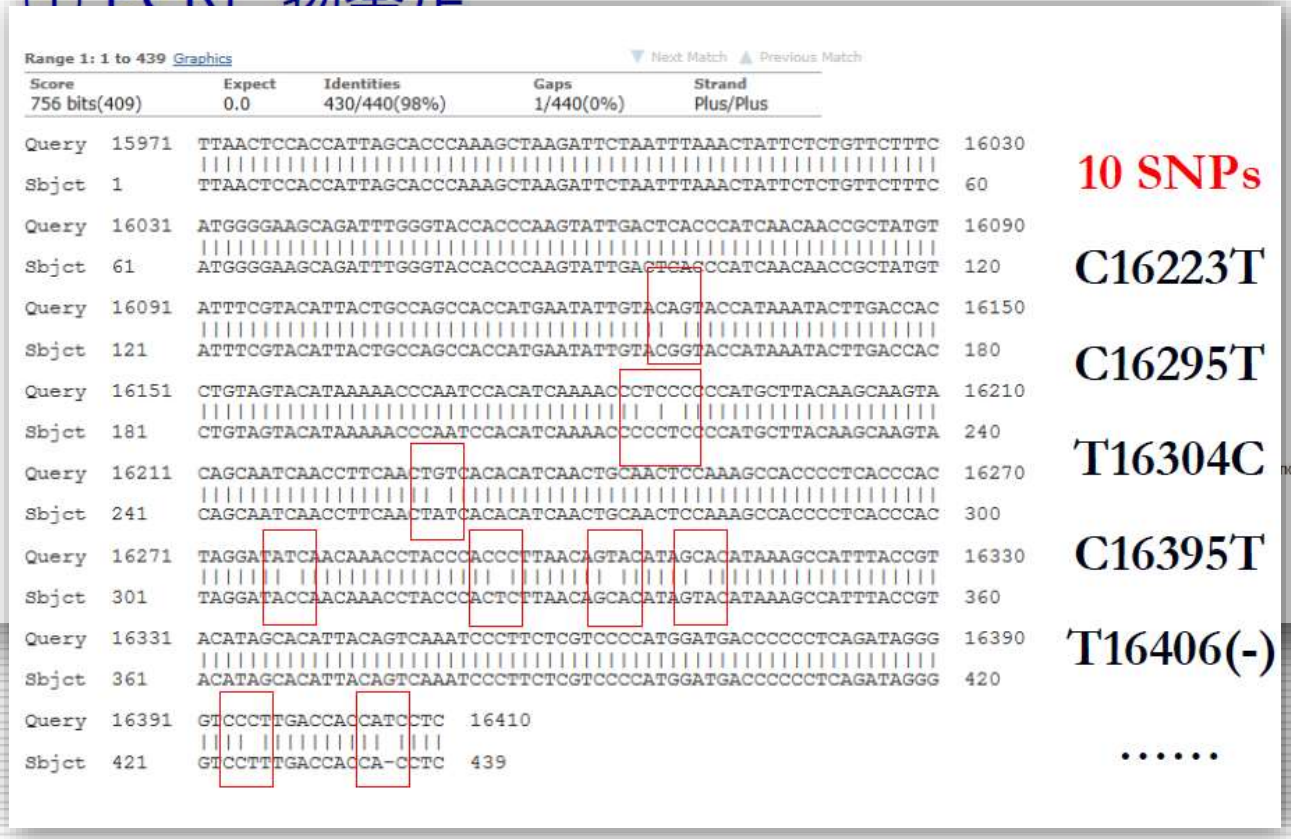
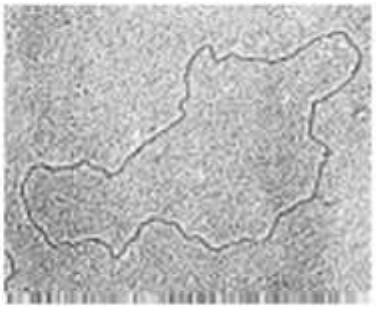

Sequencing analysis
PCR
sequencing

线粒体分子进化实验

① PCR产物鉴定

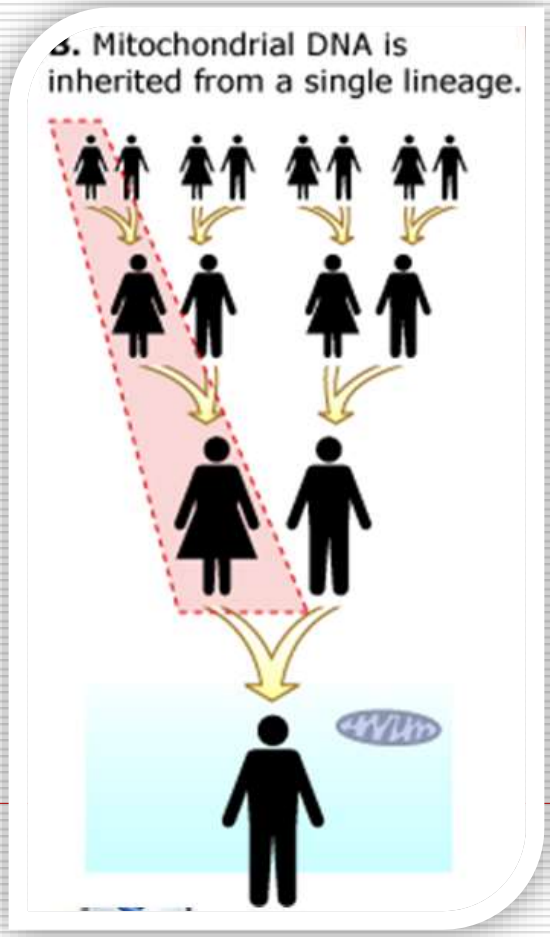


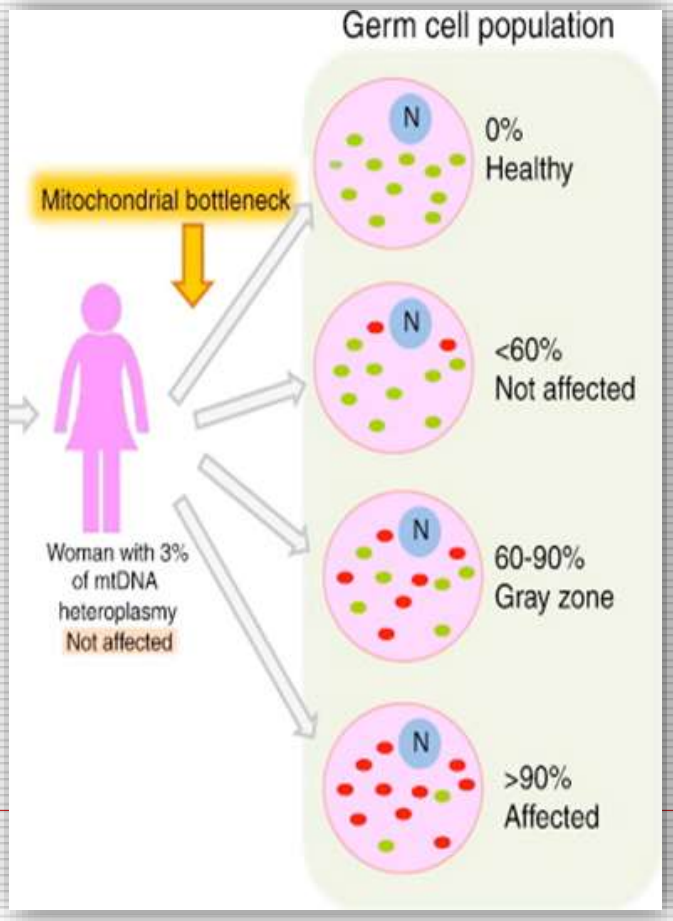
Comparison between the human nuclear and mitochondrial genomes

Chromosomes	Vs.	Mitochondrial genome
		
3 billion bp		16,500bp
Inherited from both parents		Inherited from the mother
Error –checking mechanisms		High mutation rate (10^{-4})
Mutation rate: 1 in 1,000,000,000		

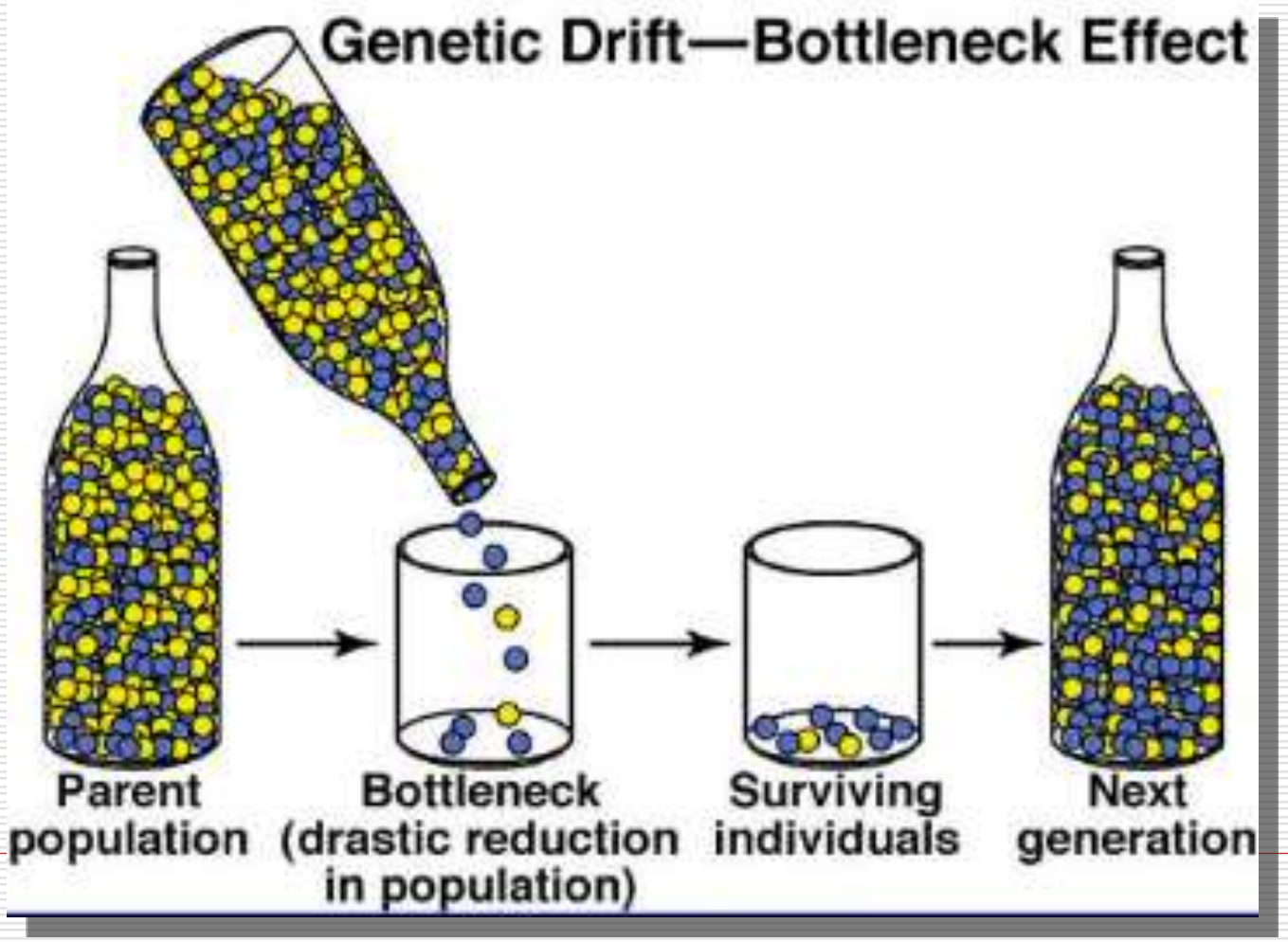
mtDNA是如何传递的?

From generation to generation：

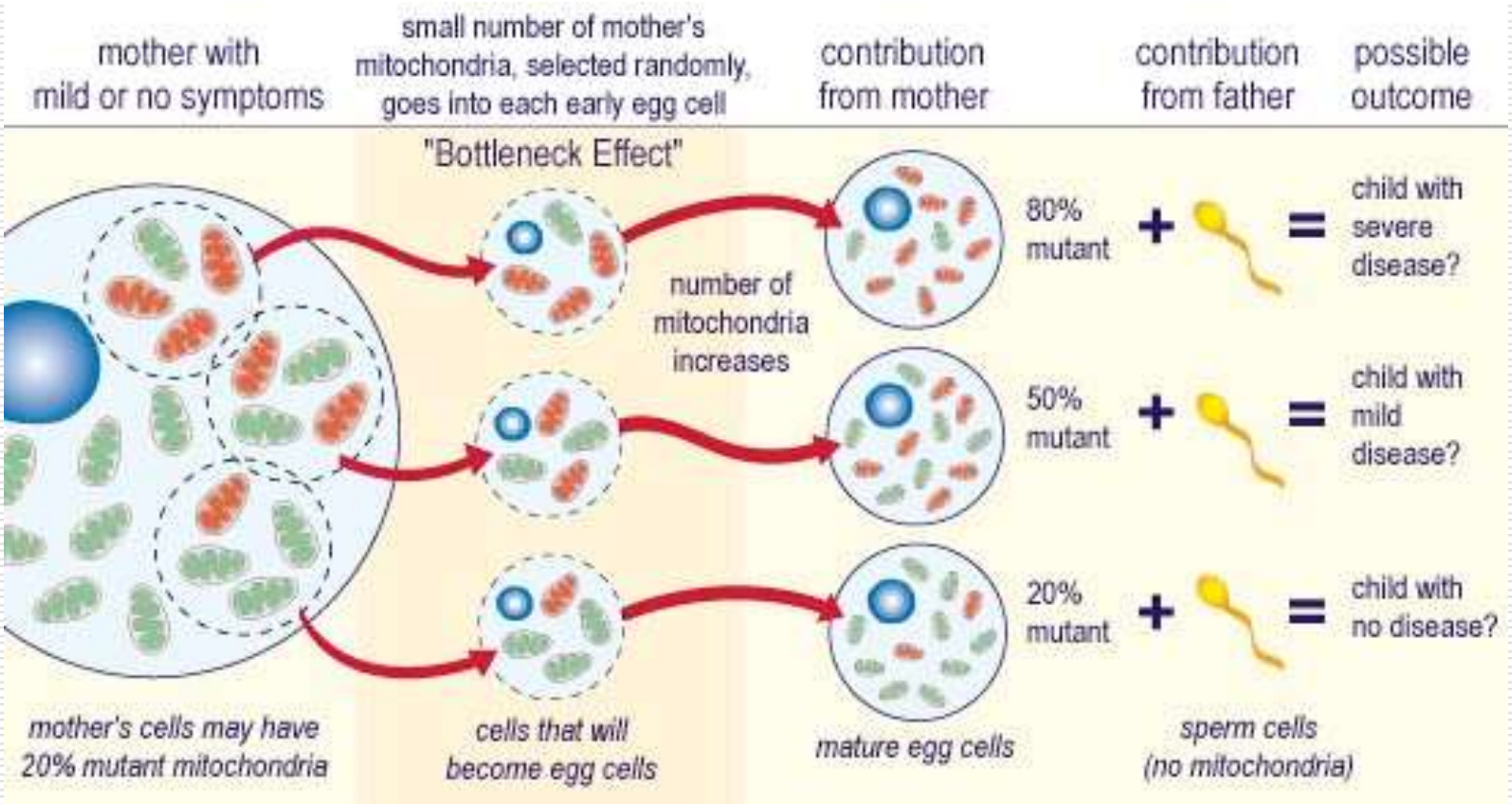




mtDNA是如何传递的?



maternal bottleneck



授课内容

一、线粒体基因组

二、线粒体基因组的遗传学基础

三、线粒体基因组与疾病

病因和发病机制

线粒体疾病由环境因素和遗传因素共同导致。这些因素导致使线粒体DNA 或 / 和 核DNA 发生基因突变，线粒体内酶功能缺陷，ATP 合成障碍，不能维持细胞的正常生理功能，产生氧化应激，使氧自由基产生增加，诱导细胞凋亡，导致疾病的发生。

一个细胞的 mt DNA 有多重拷贝，一个线粒体编码基因的表现型依赖于一个细胞内突变型和野生型 mt DNA 的相对比例，仅当突变型达到一定阈值时，病理特征才能表现出来。

线粒体基因组突变的类型

点突变 (point mutation)：病理性点突变是单一的核苷酸改变，所造成的疾病为母系遗传。同一种点突变，对不同患者可造成不同的临床表现。300多种点突变。60%影响了tRNA。

缺失 (deletion)：mt DNA 部分缺失，使基因组缩短。单发缺失多为散发性，多发缺失可呈常染色体显性或隐性遗传，提示由核 DNA 突变影响线粒体功能所致。200多种缺失

重复 (duplication)：指多余的 mt DNA 以数以千计的核苷酸插入基因组，从而使体积增大。

丢失 (depletion)：指线粒体内 mt DNA 的拷贝数减少

线粒体基因组突变修复

切除修复（Base Excision Repair, BER）：BER **主要修复尚不足以引起**DNA**螺旋变形的小量的碱基损伤。由一系列酶参加，包括**DNA**糖基化酶、**AP**内切核酸酶、**DNA**聚合酶及**DNA**连接酶等。**

重组修复（Recombination Repair, RR）：**尽管越来越多的证据显示在哺乳动物线粒体中存在重组，但**mtDNA**分子间的重组却常常不是很明显!一种可能的原因是哺乳动物线粒体的**mtDNA**分子是彼此隔离的。存在于人、酵母、黏菌的**mtDNA**的拟核限制了哺乳动物线粒体的重组率。**

线粒体遗传病

1988年，mtDNA的突变导致人类疾病的现象被首次报道。

1、nature

Nature. 1988 Feb 25;331(6158):717-9.

Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies.

Holt IJ, Harding AE, Morgan-Hughes JA.

Department of Clinical Neurology, Institute of Neurology, London, UK.

2、Science

Science. 1988 Dec 9;242(4884):1427-30.

Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ 2nd, Nikoskelainen EK.

Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

3、

Neurology. 1988 Sep;38(9):1339-46.

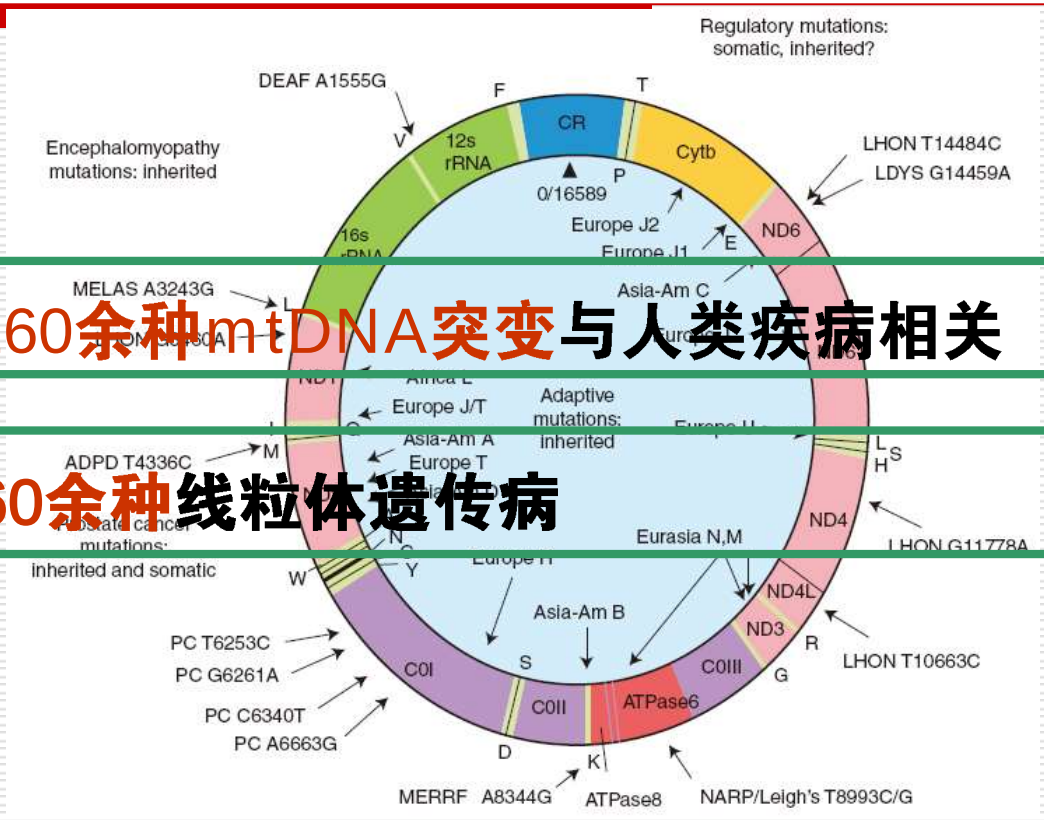
Deletions of mitochondrial DNA in Kearns-Sayre syndrome.

Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP.

Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, NY 10032-3784.

Douglas Wallace

常见线粒体遗传病



The full array of pathogenic mtDNA mutations and polymorphisms are available through Mitomap.org (MITOMAP 2012).

线粒体疾病——多系统疾病

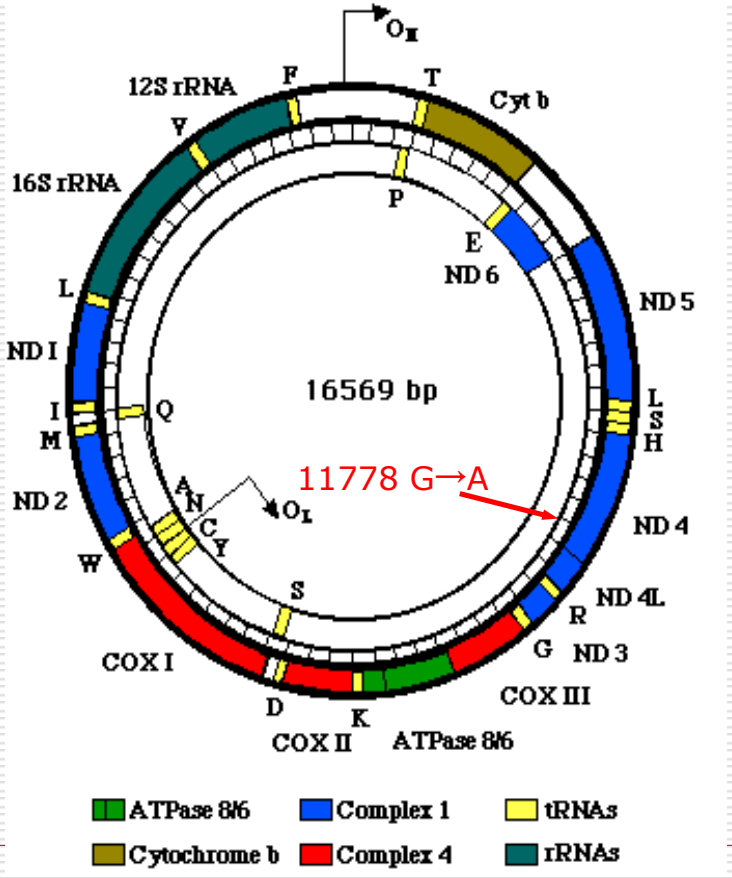


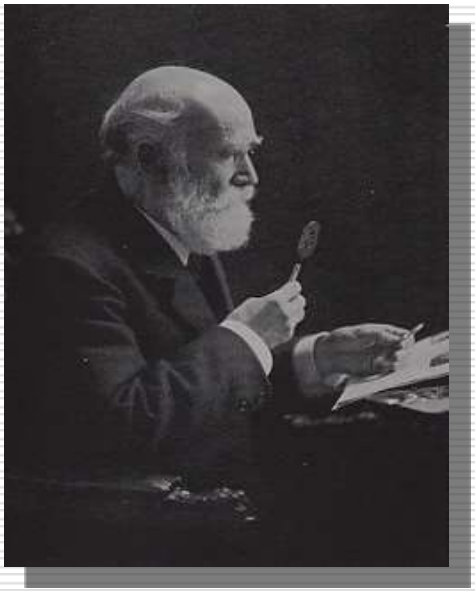
- 几乎累及机体所有器官，但耗能多的器官尤其易感。

- **每一器官都有其能量阈值效应，神经组织、骨骼肌、心脏、肾脏和肝脏对能量的依赖性依次降低，所以mtDNA突变引起的遗传病经常表现出神经组织和肌肉组织的异常。**

(一) Leber遗传性视神经病 (LHON)

（Leber Hereditary Optic Neuropathy，LHON）





LHON是以德国眼科医生Theodor Leber（1840～1917）的名字命名的，为一种急性或亚急性发作的母系遗传病。男女病人比例4:1

临床表现

1、典型的首发症状为视中心盲点的出现，患者视物模糊，接着在几个月内出现无痛性、完全或接近完全失明。

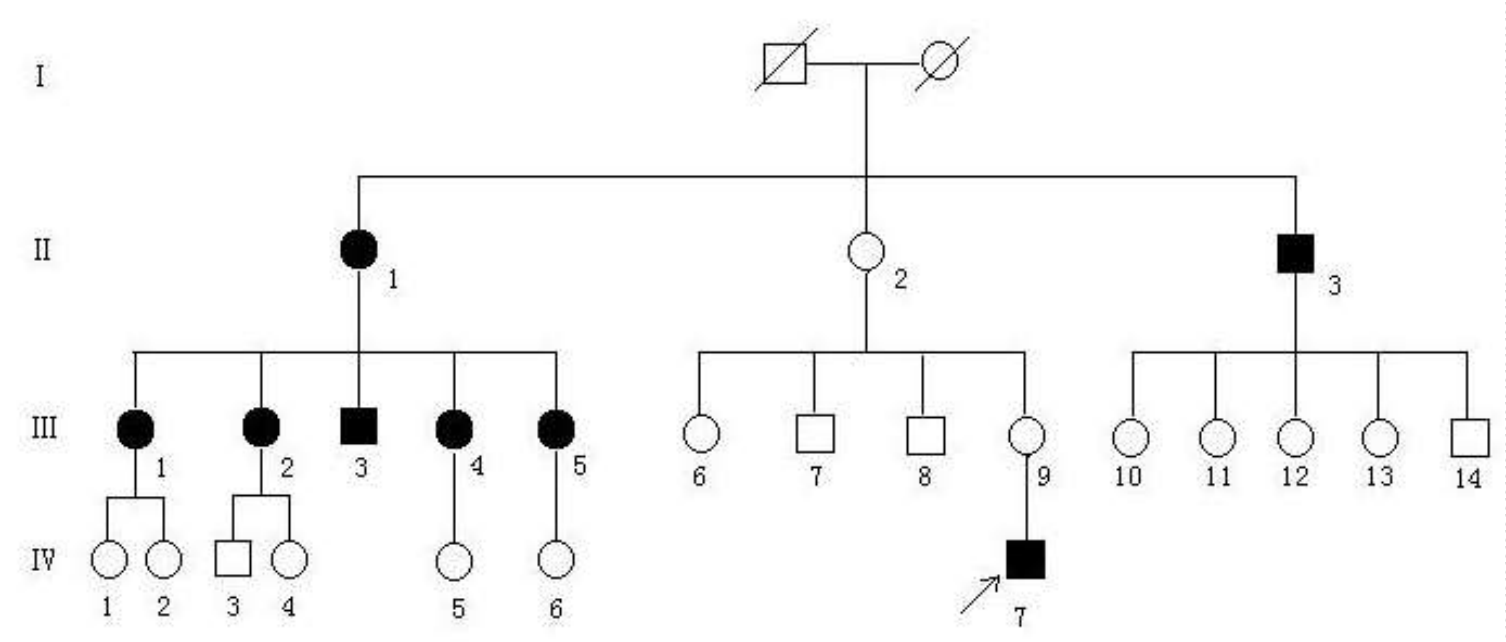
临床表现

2、两眼同时或先后发病，可间隔数天或数月，一般不超过一年。

3、发病年龄4-70岁，多在青春期（18-23岁）发病，倾向于男性发病。是目前世界上**最常见**的**青壮年致盲性疾病之一**。

4、本病偶可并发神经系统、心脏、骨骼肌改变等，如头痛、小脑性共济失调、预激综合征、肌张力障碍等。

一个LHON的典型系谱



- Leber遗传性视神经病是母系遗传的疾病。至今尚未发现一个男性患者将此病传给后代。
- 先症者母亲没有病?

发病机制

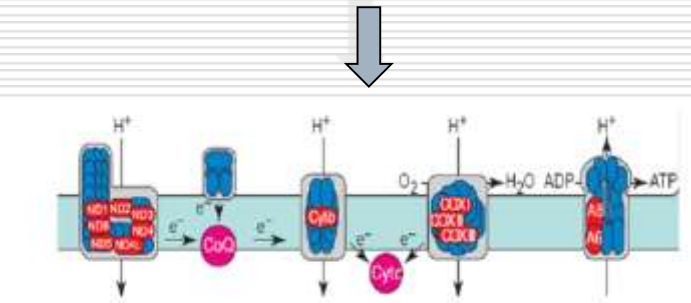
在9种编码线粒体蛋白的基因（ND1, ND2, CO1, ATP6, CO3, ND4、ND5、ND6、CYTB）**种，至少有18种错义突变直接或间接地导致**LHON。

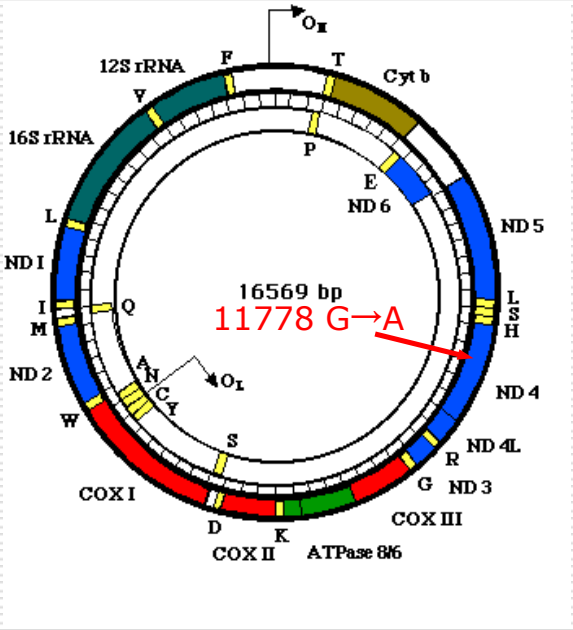
主要突变类型：

MTND1*LHON3460A

MTND4*LHON11778A(50%-70%)

MTND6*LHON14484C





ND1,ND4,ND6等的基因突变

↓

使呼吸链ComplexI中的NADH脱氢酶活性降低

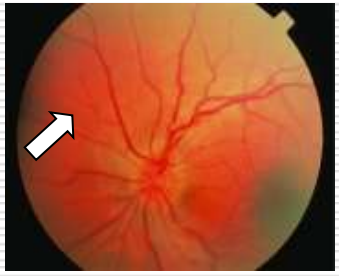
↓

线粒体产能下降

↓

需能量多的视神经组织损害最大

↓



↓

视神经萎缩，失明

(二)线粒体脑肌病: MERRF综合征

—肌阵挛性癫痫和破碎红纤维病

（ Myoclonus Epilepsy With Ragged Red Fibers, MERRF ）

•任何类型的用力都会引起疲劳。有时早上起床的时候也觉得没力气……

•只是简单的日常活动都会引起疲劳。

•What is the weakness like? Well, strap 160 pounds on your body. Walk around like that, all day, every day.

临床表现

1、**轻微的肌病**：肌病通常是轻微的，有时运动不能耐受突出。

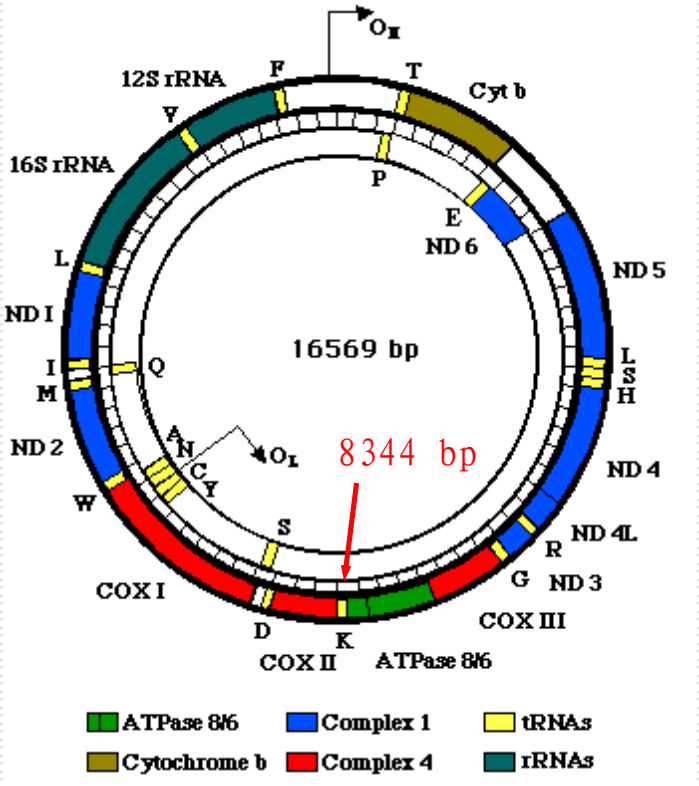
2、**皮质肌阵挛**：影响头部、四肢及躯干，可以是自发的、反射性的或者动作诱发。也可能出现局灶性癫痫发作。

3、**共济失调**：进行性共济失调及其它的小脑症状可能是轻微的或者是致残的主要原因。

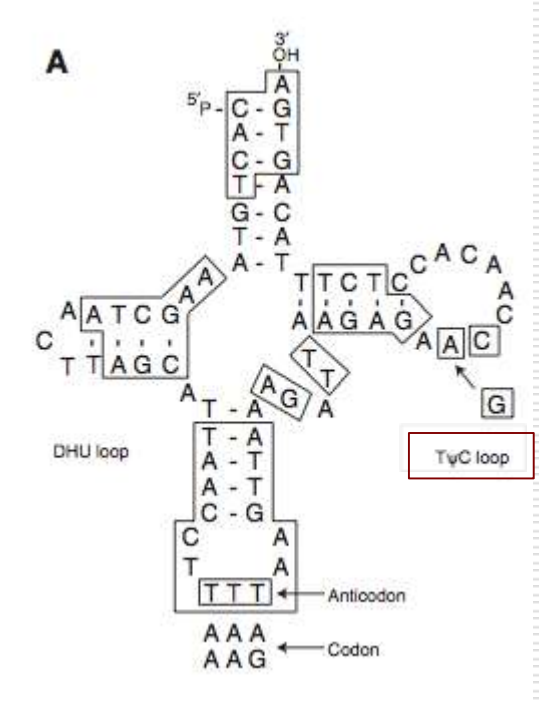
4、**认知障碍**。

其它一些常见的表现包括：感觉神经性听力丧失、周围神经病变、身材矮小、乳酸酸中毒等。

发病机制
(MTTK*MERRF8344G)



tRNA^{lys} nt8344 A >G



发病机制

突变使转运lys的tRNA功能受损

↓

导致呼吸链mtDNA编码的蛋白质翻译过程障碍

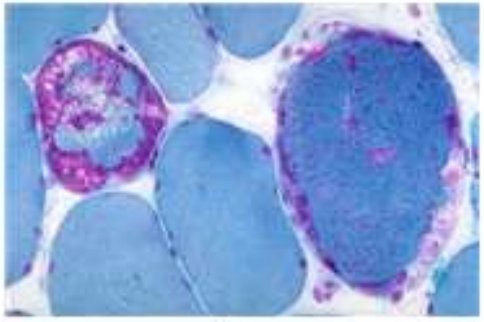
↓

使部分呼吸链复合物合成障碍

↓

异常线粒体

（聚集于肌细胞，特异染料着染成红色，出现碎红纤维）

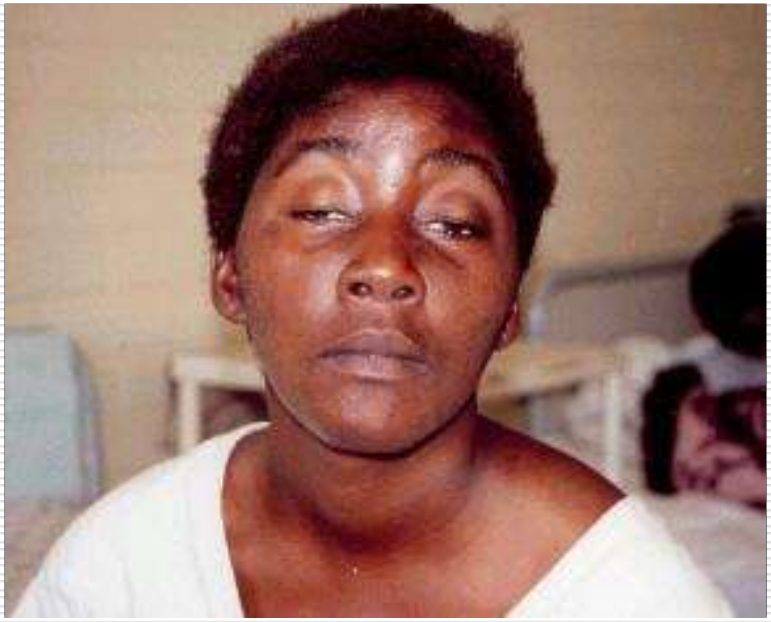


↓

以肌无力，肌运动失调等“肌病”为主的综合症

(三)线粒体脑肌病: KSS综合征

(Keams Sayre Syndrome，KSS)



临床表现：

进行性外部眼肌麻痹

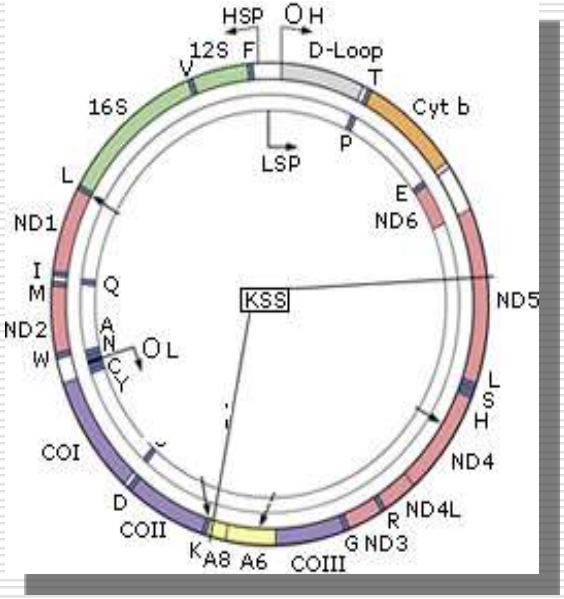
视网膜色素变性

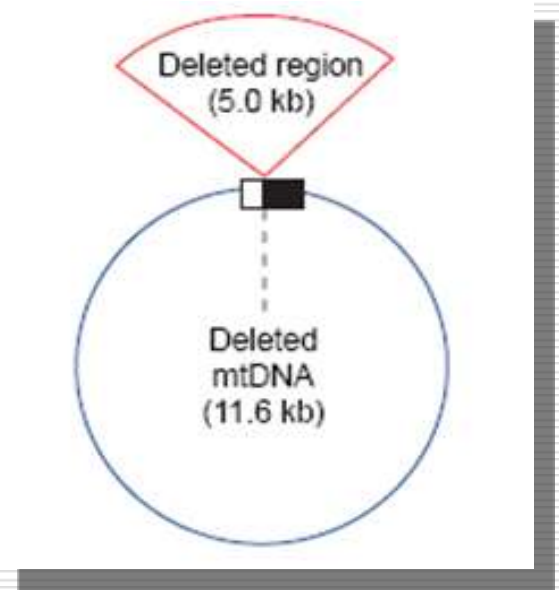
心肌电传导障碍

小脑共济失调

散发

发病机制





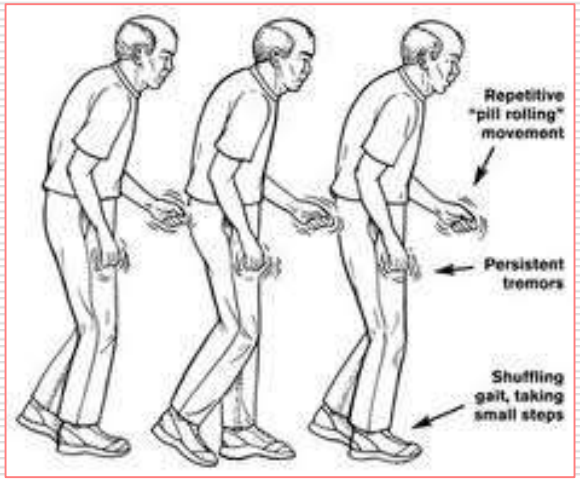
最常见：8470～13447；8673～16073

肌细胞中突变的线粒体大于85%

(四) MtDNA突变与帕金森病

帕金森病（Parkinson disease）：1817**年被英国药剂师**James Parkinson (1755-1824)**报道**.

晚年发病的运动失调症。有震颤，动作迟缓且常常错误等症状，又称震颤性麻痹。少数患者有痴呆症状。患者脑组织，特别是灰质中存在mtDNA**缺失。**



发病机制 1

环境因素：

经常接触杀虫剂、除草剂、工业化学制品、木质纸浆；

长期居住在乡村、经常接触冷水、从事农业耕作；

痕量的金属、氰化物、油漆的稀释剂（香蕉水）、CO、CS2等；

有毒化合物，如四羟基奎宁和β–咔啉。

MPTP(1-甲基–4–苯基–1,2,3,6–四氢吡啶）

——杜冷丁合成过程的衍生副产物，用于PD模型建立

MPTP作用于星形胶质细胞，经单胺氧化酶催化形成MPP+并特异性破坏多巴胺能神经元的线粒体复合物I，引起PD 症状。

发病机制 2

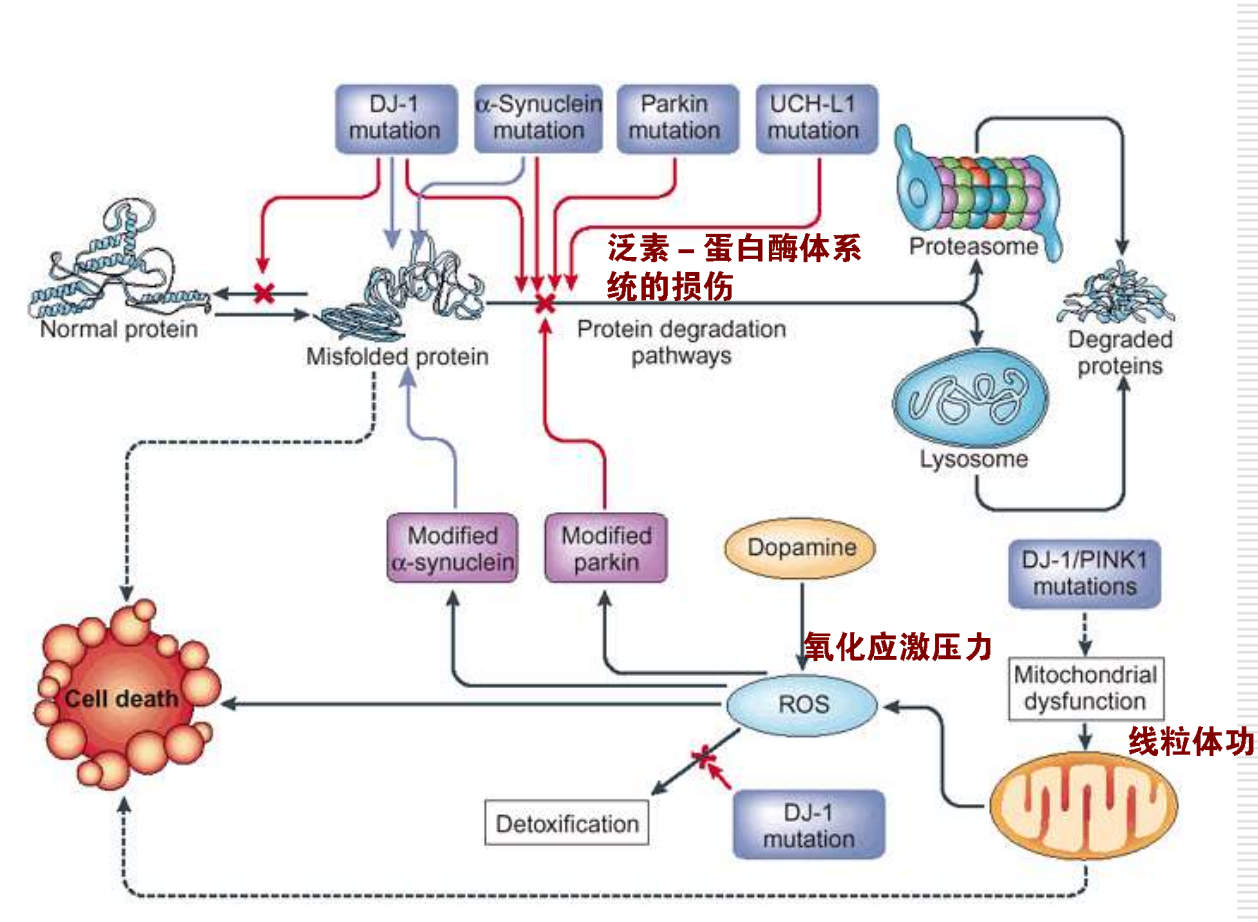
遗传因素： 5～10%的PD患者是家族性PD，常染色体显性（AD）或隐性遗传（AR）。Parkin 、PINK、DJ-1和UCH-L1的突变与PD发病相关。

Table 1 Genes and loci linked to familial PD

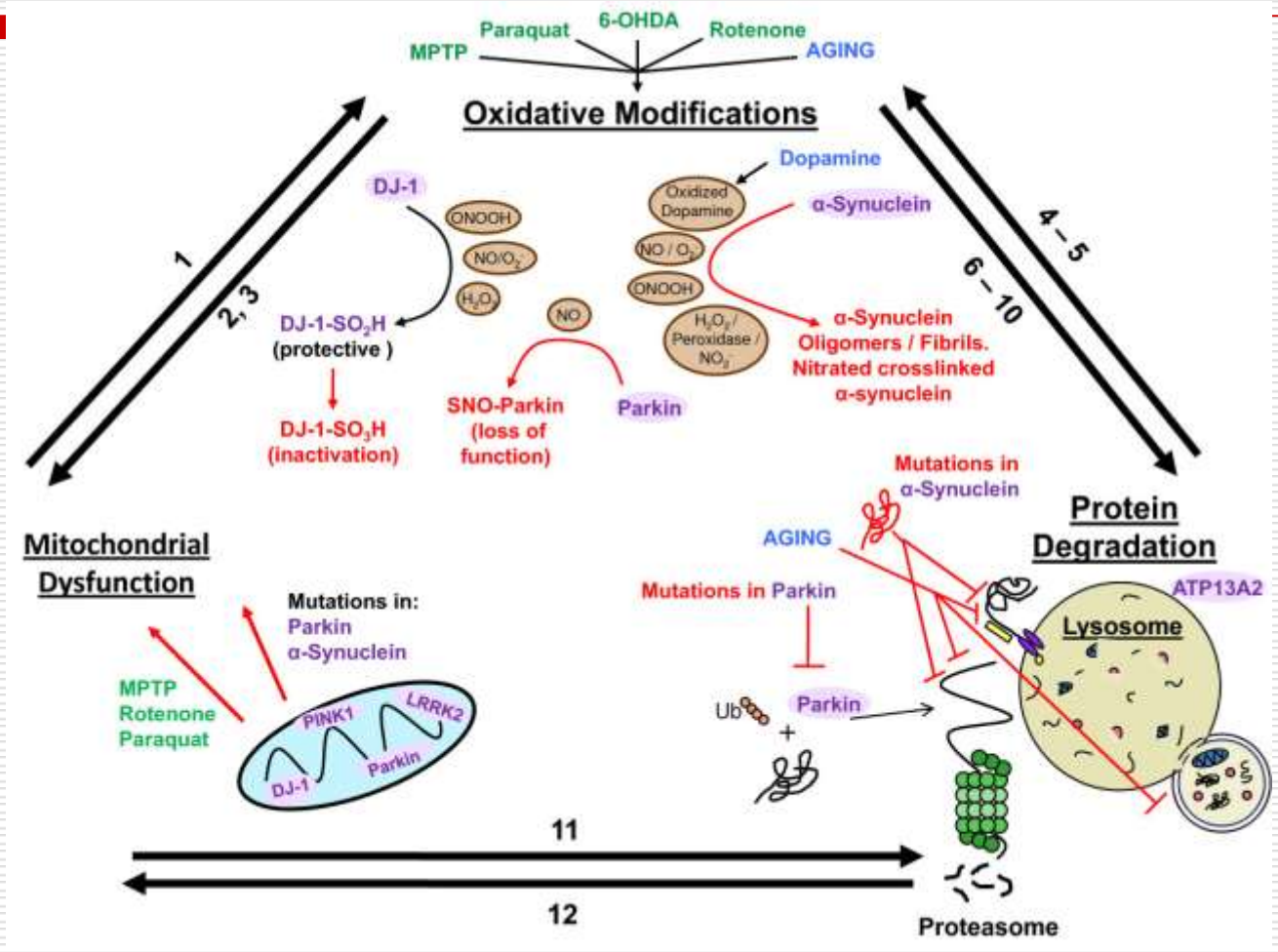
Locus	Chromosomal location	Protein	Inheritance pattern	Atypical PD features	Lewy bodies
PARK1	4q21	α-Synuclein[a]	AD	Early onset Lower prevalence of tremor	Yes
PARK2	6q25.2–q27	Parkin	AR	Early or juvenile onset More frequent dystonia and levodopa-induced dyskinesias Slower disease progression	Mostly negative[b]
PARK3	2p13	Unknown	AD	Dementia in some individuals Rapid progression	Yes
PARK4[c]	4p15	Unknown	AD	Early onset Rapid progression Dementia Autonomic dysfunction Postural tremor	Yes
PARK5	4p14	UCH-L1	AD	None	Unknown
PARK6	1p36	PINK1	AR	Early onset Slow progression	Unknown
PARK7	1p36	DJ-1	AR	Early onset Psychiatric symptoms Slow progression	Unknown
PARK8	12p11.2–q13.1	Unknown	AD	None	No
PARK9	1p36	Unknown	AR	Juvenile onset Spasticity Supranuclear gaze paralysis Dementia	Unknown

AD, autosomal dominant; AR, autosomal recessive. [a]Including mutations and wild-type multiplications. [b]Lewy bodies reported in one individual with parkin mutations[54]. [c]The initial PARK4 linkage to 4p15 could not be confirmed, and the PD phenotype in this family was subsequently linked to a PARK1 variant (α-synuclein triplication)[11].

Genetic mutations and the pathogenesis of PD

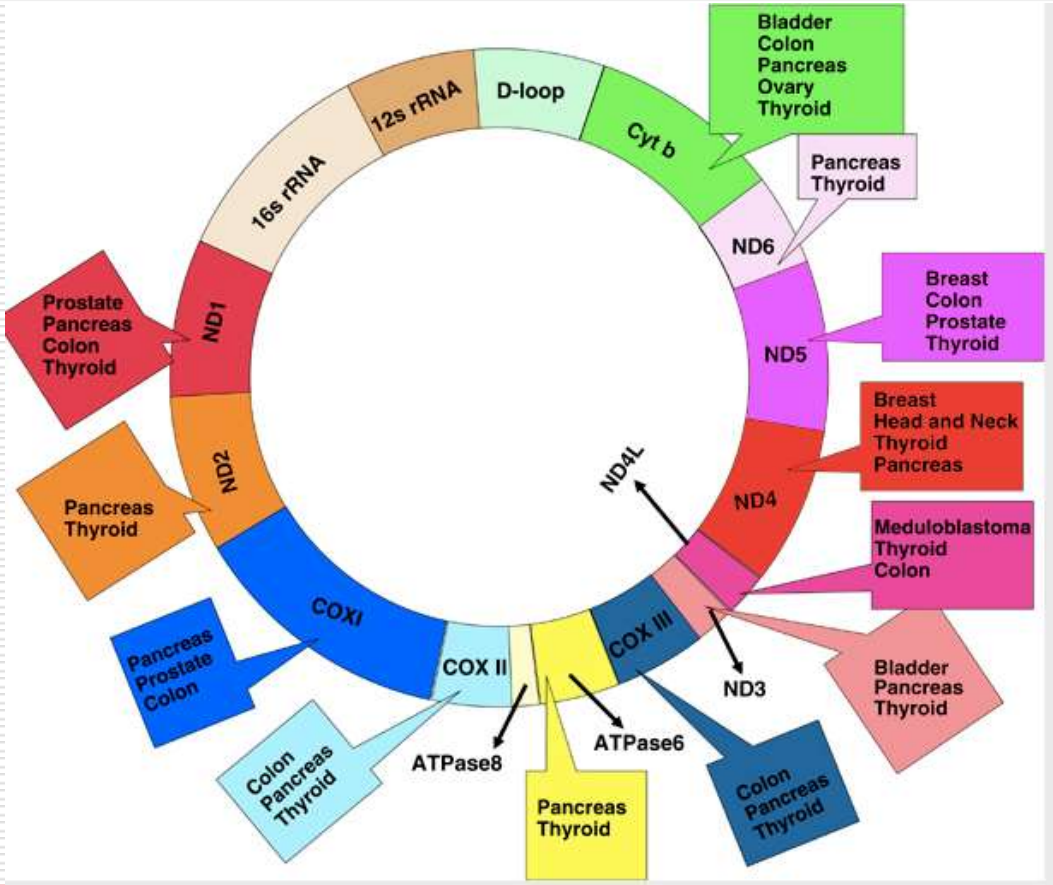


A "Bermuda Triangle" in PD



(五) 线粒体功能障碍与肿瘤

肿瘤的生物学特征不仅取决于核内遗传物质，而且与核外的mtDNA密切相关。在多种肿瘤（膀胱癌、头颈肿瘤、肝癌、结肠癌、肾癌、乳腺癌、前列腺癌、血液系统肿瘤等）中，相继发现：mtDNA**异常、线粒体结构与功能异常**，从而导致肿瘤细胞能量代谢障碍。



发病机制 1

mtDNA mutations

1. Mutations in complex I
2. Mutations in complex III
3. Mutations in complex IV
4. Mutations in complex V

Region	Nucleotide change	Nucleotide position	Type of cancer	Amino-acid change	Reference
ND1	G-A	3434	Prostate	CYS-TYR	Jeronimo et al. (2001)
ND1	A-G	3505	Prostate, Pancreatic	THR-ALA	Jeronimo et al. (2001); Jones et al. (2001)

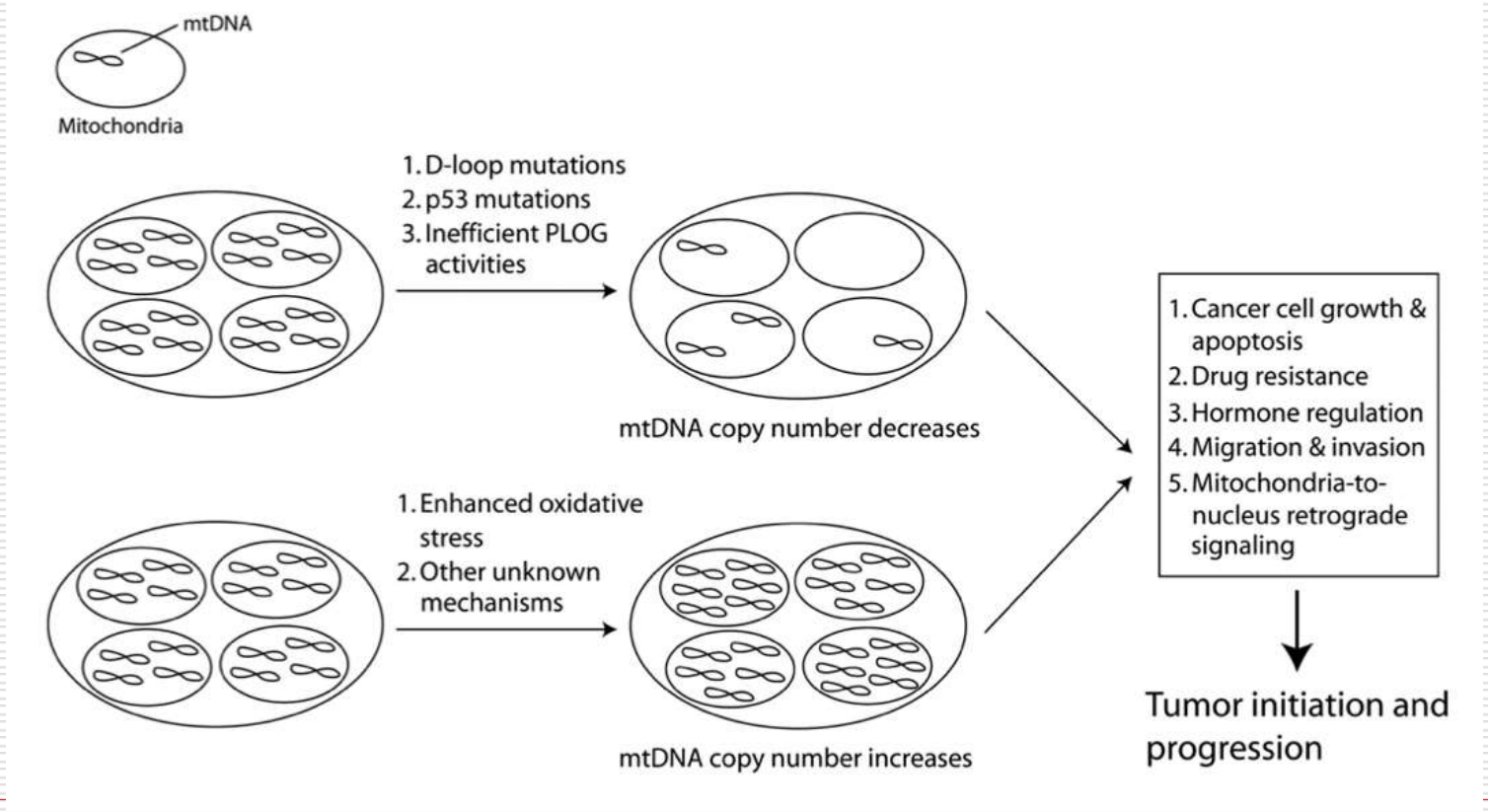
Region	Nucleotide change	Nucleotide position	Type of cancer	Amino-acid change	References
CYT B	7 aa deletion	15 642	Bladder		Fliss et al. (2000)
CYT B	G-A	14 985	Colon	ARG-GLN	Polyak et al. (1998)
CYT B	T-C	15 572	Colon	PHE-LEU	Polyak et al. (1998)
CYT B	G-C	15 884	Pancreatic	ALA-PRO	Jones et al. (2001)
CYTB	G-A	15 761	Ovarian	GLY-Stop	Liu et al. (2001)
CYTB	Deletion	14 927–14 941	Thyroid		Maximo et al. (2002)
CYTB	A-G	15 182	Thyroid	ILE-VAL	Maximo et al. (2002)

Region	Nucleotide change	Nucleotide position	Type of cancer	Amino-acid change	References
COX 1	G-A	5973	Pancreatic, Prostate	ALA-THR	Jeronimo et al. (2001); Jones et al. (2001)
COX 1	G-A	6267	Pancreatic, Prostate	ALA-THR	Jeronimo et al. (2001); Jones et al. (2001)
COX 1	G-A	5913	Prostate	ASP-ASN	Jeronimo et al. (2001)
COX 1	G-A	6264	Colon	GLY-Stop	Polyak et al. (1998)
COX 1	A-G	5935	Prostate	ASN-SER	Jeronimo et al. (2001)
COX 1	G-A	5949	Prostate	GLY-Stop	Jeronimo et al. (2001)
COX 1	G-A	6081	Prostate	ALA-THR	Jeronimo et al. (2001)
COX 1	G-A	6150	Prostate	VAL -ILE	Jeronimo et al. (2001)
COX 1	T-C	6124	Prostate	MET-THR	Jeronimo et al. (2001)
COX 1	T-C	6253	Prostate	MET-THR	Jeronimo et al. (2001)
COX 1	G-A	6261	Prostate	ALA-THR	Jeronimo et al. (2001)
COX 1	G-A	6285	Prostate	VAL-ILE	Jeronimo et al. (2001)
COX 1	C-T	6340	Prostate	THR-ILE	Jeronimo et al. (2001)
COX 1	G-A	6480	Prostate	VAL-ILE	Jeronimo et al. (2001)
COX 1	A-G	6663	Prostate	ILE-VAL	Jeronimo et al. (2001)
COX 1	G-T	6924	Prostate	ALA-SER	Jeronimo et al. (2001)
COX 1	G-A	7041	Prostate	VAL-ILE	Jeronimo et al. (2001)
COX 1	T-C	7080	Prostate	PHE-LEU	Jeronimo et al. (2001)
COX 1	A-G	7083	Prostate	ILE-VAL	Jeronimo et al. (2001)
COX 1	A-G	7158	Prostate	ILE-VAL	Jeronimo et al. (2001)
COX 1	A-C	7305	Prostate	MET-LEU	Jeronimo et al. (2001)
COX II	G-A	8009	Colon	VAL-MET	Polyak et al. (1998)
COX II	G-T	7986	Pancreatic	ARG-GLN	Jones et al. (2001)
COX II	Deletion	7631–8203	Thyroid		Maximo et al. (2002)
COX II	Deletion	7627–8195	Thyroid		Maximo et al. (2002)

Region	Nucleotide change	Nucleotide position	Cancer type	Amino acid change	Ref number
ATPase6	T-C	8996	Pancreatic	MET-THR	Jones *et al.* (2001)
ATPase6	T-G	9070	Pancreatic	SER-ALA	Jones *et al.* (2001)
ATPase6	A-G	8701	Thyroid	THR-ALA	Maximo *et al.* (2002)
ATPase6	T-C	9137	Thyroid	ILE-THR	Maximo *et al.* (2002)
ATPase6	A-G	8716	Thyroid	LYS-GLU	Maximo *et al.* (2002)

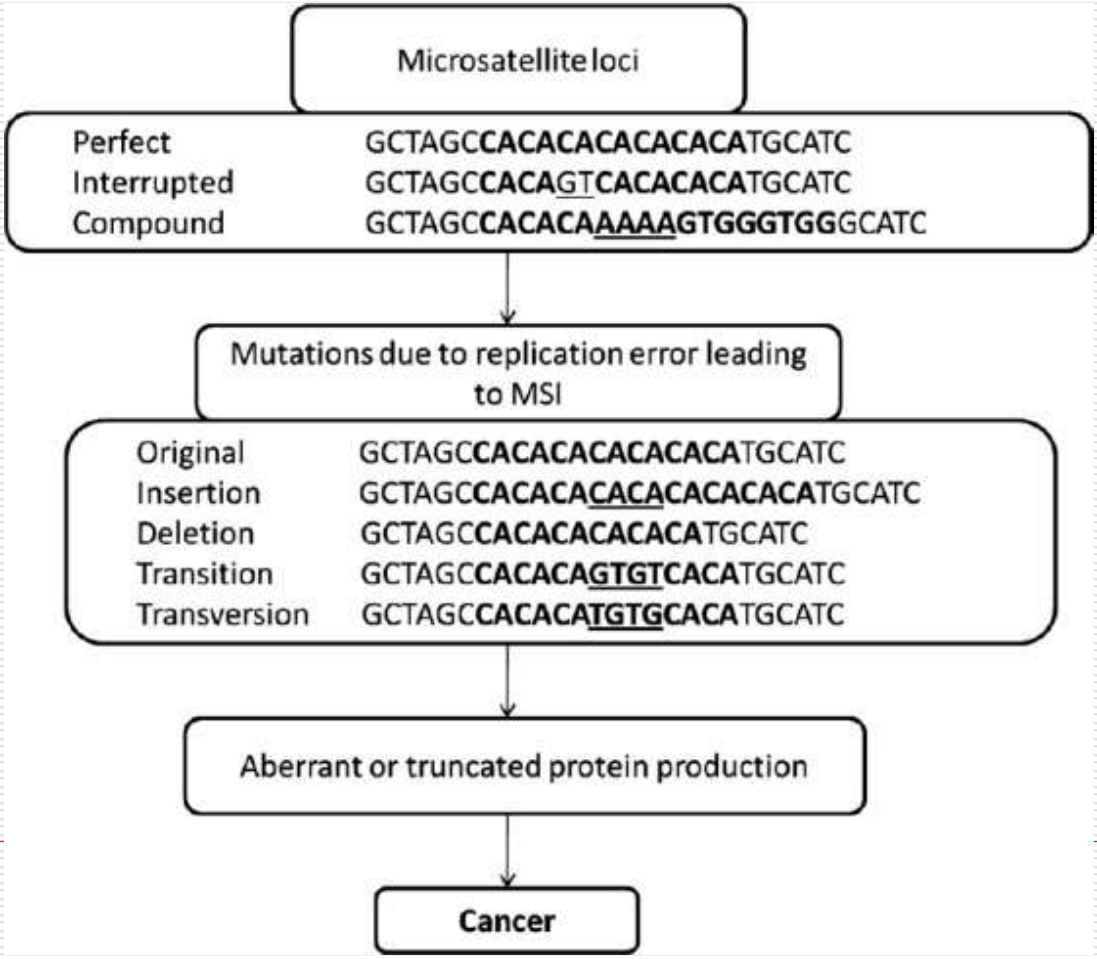
发病机制 2

mtDNA copy number alterations



发病机制 3

mtDNA Microsatellite Instability



发病机制 4

OPEN ACCESS
CellPress

Cell Reports
Article

A Mitochondrial Switch Promotes Tumor Metastasis

Paolo E. Porporato,[1] Valéry L. Payen,[1] Jhudit Pérez-Escuredo,[1] Christophe J. De Saedeleer,[1] Pierre Danhier,[2] Tamara Copetti,[1] Suveera Dhup,[1] Morgane Tardy,[1] Thibaut Vazeille,[1] Caroline Bouzin,[1] Olivier Feron,[1] Carine Michiels,[3] Bernard Gallez,[2] and Pierre Sonveaux[1,*]

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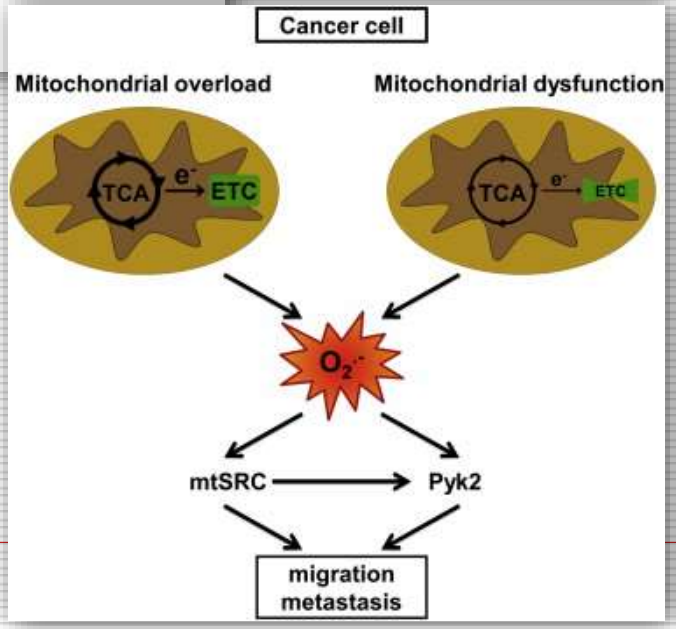
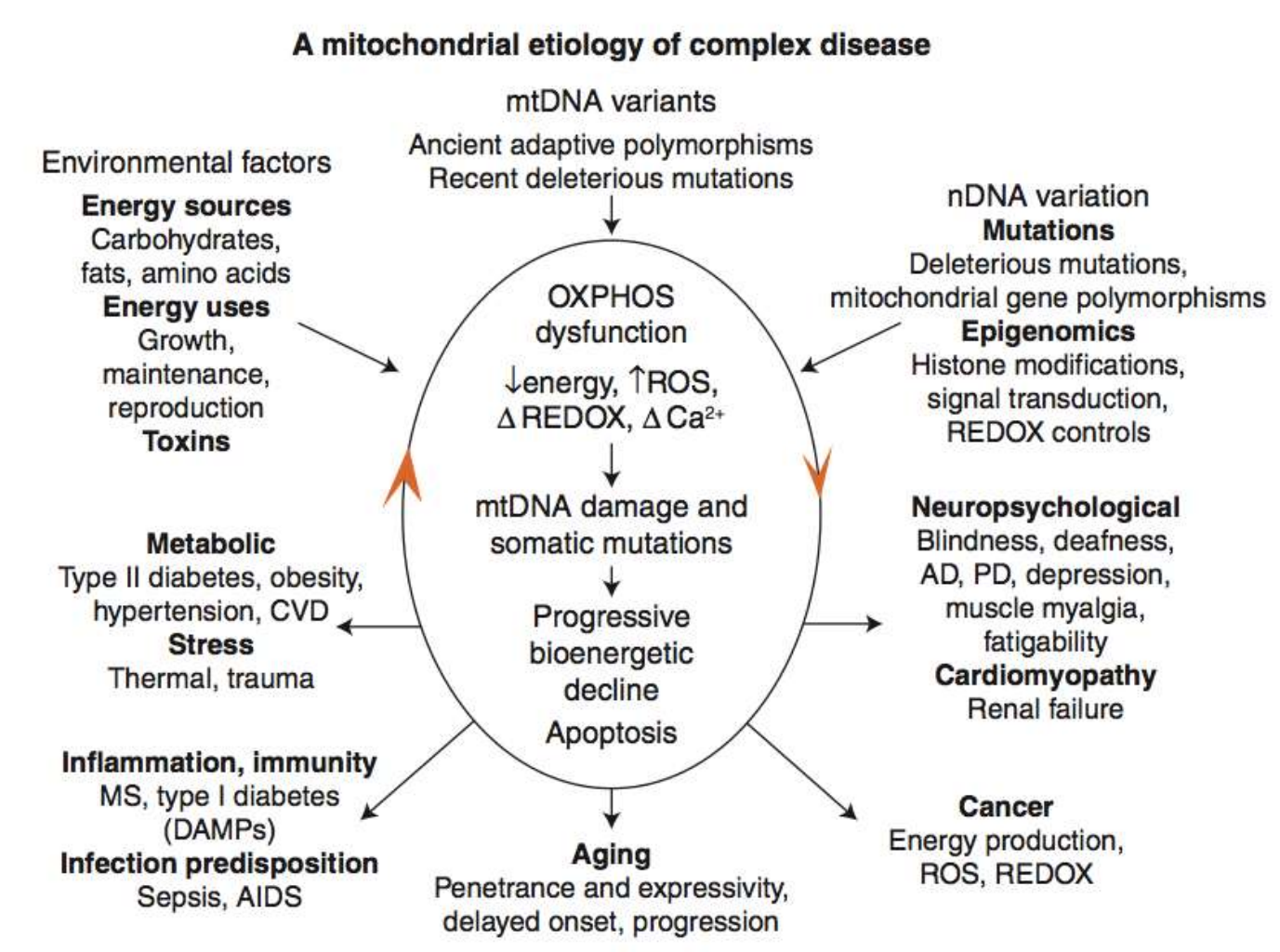
http://dx.doi.org/10.1016/j.celrep.2014.06.043



A mitochondrial etiology of complex disease



线粒体自噬对帕金森氏症的影响（Nature , 2015 ）

ARTICLE

doi:10.1038/nature13418

The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy

Baris Bingol[1]*, Joy S. Tea[1]*, Lilian Phu[2], Mike Reichelt[3], Corey E. Bakalarski[4], Qinghua Song[5], Oded Foreman[3], Donald S. Kirkpatrick[2] & Morgan Sheng[1]

Cells maintain healthy mitochondria by degrading damaged mitochondria through mitophagy; defective mitophagy is linked to Parkinson's disease. Here we report that USP30, a deubiquitinase localized to mitochondria, antagonizes mitophagy driven by the ubiquitin ligase parkin (also known as PARK2) and protein kinase PINK1, which are encoded by two genes associated with Parkinson's disease. Parkin ubiquitinates and tags damaged mitochondria for clearance. Overexpression of USP30 removes ubiquitin attached by parkin onto damaged mitochondria and blocks parkin's ability to drive mitophagy, whereas reducing USP30 activity enhances mitochondrial degradation in neurons. Global ubiquitination site profiling identified multiple mitochondrial substrates oppositely regulated by parkin and USP30. Knockdown of USP30 rescues the defective mitophagy caused by pathogenic mutations in parkin and improves mitochondrial integrity in parkin- or PINK1-deficient flies. Knockdown of USP30 in dopaminergic neurons protects flies against paraquat toxicity *in vivo*, ameliorating defects in dopamine levels, motor function and organismal survival. Thus USP30 inhibition is potentially beneficial for Parkinson's disease by promoting mitochondrial clearance and quality control.

线粒体DNA损伤引发抗病毒固有免疫反应（Nature）

LETTER

doi:10.1038/nature14156

Mitochondrial DNA stress primes the antiviral innate immune response

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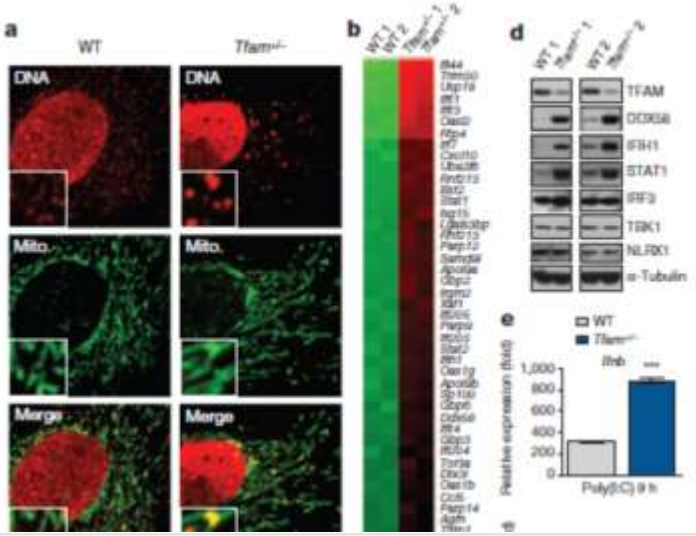
Mitochondrial DNA (mtDNA) is normally present at thousands of copies per cell and is packaged into several hundred higher-order structures termed nucleoids[1]. The abundant mtDNA-binding protein TFAM (transcription factor A, mitochondrial) regulates nucleoid architecture, abundance and segregation[2]. Complete mtDNA depletion profoundly impairs oxidative phosphorylation, triggering calcium-dependent stress signalling and adaptive metabolic responses[3]. However, the cellular responses to mtDNA instability, a physiologically relevant stress observed in many human diseases and ageing, remain poorly defined[4]. Here we show that moderate mtDNA stress elicited by TFAM deficiency engages cytosolic antiviral signalling to enhance the expression of a subset of interferon-stimulated genes. Mechanistically, we find that aberrant mtDNA packaging promotes escape of mtDNA into the cytosol, where it engages the DNA sensor cGAS (also known as MB21D1) and promotes STING (also known as TMEM173)–IRF3-dependent signalling to elevate interferon-stimulated gene expression, potentiate type I interferon responses and confer broad viral resistance. Furthermore, we demonstrate that herpesviruses induce mtDNA stress, which enhances antiviral signalling and type I interferon responses during infection. Our results further demonstrate that mitochondria are central participants in innate immunity, identify mtDNA stress as a cell-intrinsic trigger of antiviral signalling and suggest that cellular monitoring of mtDNA homeostasis cooperates with canonical virus sensing mechanisms to fully engage antiviral innate immunity.

in *Tfam*$^{+/-}$ MEFs validated the microarray results (Fig. 1c, d). Finally, *Tfam*$^{+/-}$ MEFs expressed three- to fourfold more *Ifnb* and *Ifna4* upon transfection with the IFIH1 agonist poly(I:C) (Fig. 1e), consistent with enhanced type I interferon responses.



线粒体在肿瘤免疫杀伤中的新作用（Nature commu . 2015）

nature COMMUNICATIONS

ARTICLE

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Regulation of NKT cell-mediated immune responses to tumours and liver inflammation by mitochondrial PGAM5-Drp1 signalling

Young Jun Kang[1], Bo-Ram Bang[1], Kyung Ho Han[2], Lixin Hong[1], Eun-Jin Shim[1], Jianhui Ma[1], Richard A. Lerner[2] & Motoyuki Otsuka[3]

The receptor-interacting protein kinase 3 (RIPK3) plays crucial roles in programmed necrosis and innate inflammatory responses. However, a little is known about the involvement of RIPK3 in NKT cell-mediated immune responses. Here, we demonstrate that RIPK3 plays an essential role in NKT cell function via activation of the mitochondrial phosphatase phosphoglycerate mutase 5 (PGAM5). RIPK3-mediated activation of PGAM5 promotes the expression of cytokines by facilitating nuclear translocation of NFAT and dephosphorylation of dynamin-related protein 1 (Drp1), a GTPase is essential for mitochondrial homoeostasis. *Ripk3*$^{-/-}$ mice show reduced NKT cell responses to metastatic tumour cells, and both deletion of RIPK3 and pharmacological inhibition of Drp1 protects mice from NKT cell-mediated induction of acute liver damage. Collectively, the results identify a crucial role for RIPK3-PGAM5-Drp1/NFAT signalling in NKT cell activation, and further suggest that RIPK3-PGAM5 signalling may mediate crosstalk between mitochondrial function and immune signalling.

高脂饮食或是线粒体代谢疾病患者的福音?

PNAS

High-fat diet and FGF21 cooperatively promote aerobic thermogenesis in mtDNA mutator mice

Christopher E. Wall[a,b], Jamie Whyte[a], Jae M. Suh[a], Weiwei Fan[a], Brett Collins[a], Christopher Liddle[c], Ruth T. Yu[a], Annette R. Atkins[a], Jane C. Naviaux[d], Kefeng Li[d], Andrew Taylor Bright[d], William A. Alaynick[d], Michael Downes[a], Robert K.

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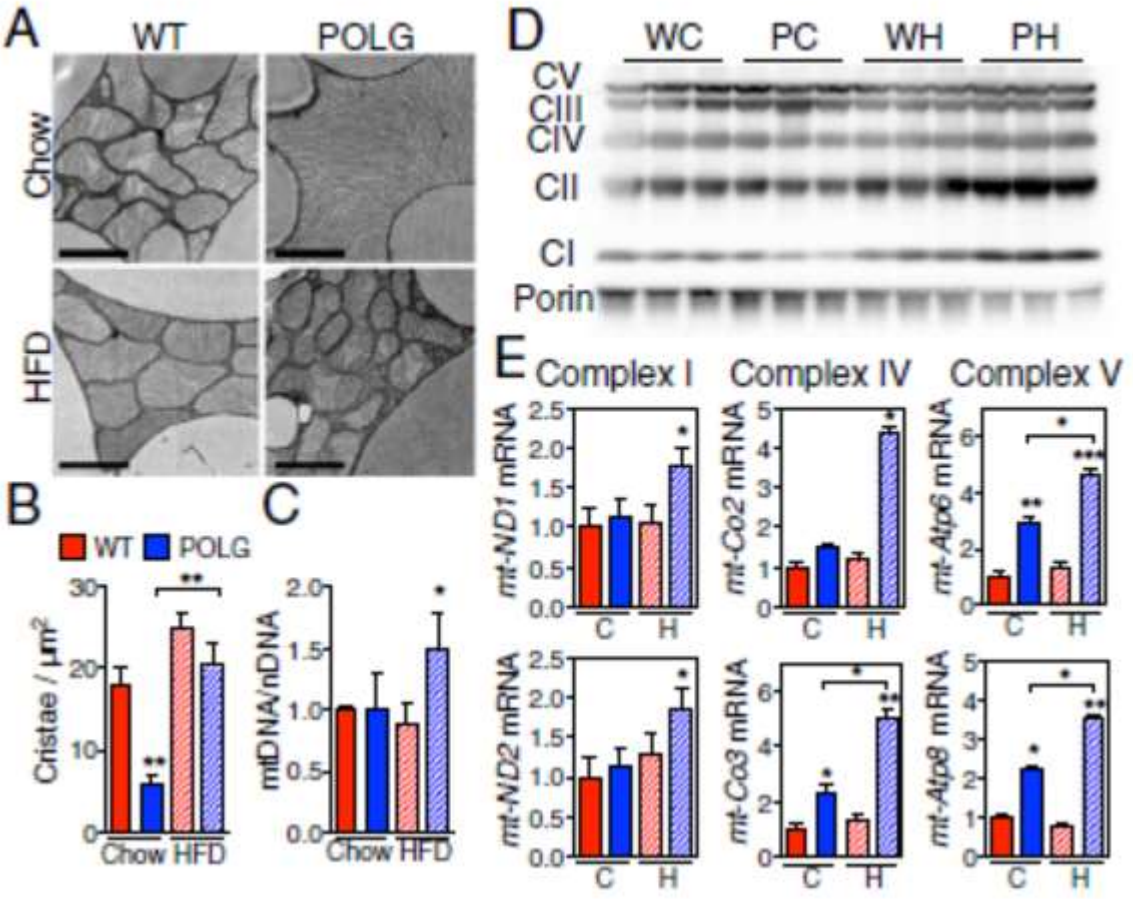
ifornia at San Diego
and [f]Howard Hughes

Contributed

unichiro Sonoda)



RESEARCH

RESEARCH ARTICLE

HEART MITOCHONDRIA

Imbalanced OPA1 processing
mitochondrial fragmentation
heart failure in mice

Timothy Wai,[1,2]* Jaime García-Prieto,[3]* Michael J. Baker,[1] Carsten
Paule Benit,[4,5] Pierre Rustin,[4,5] Francisco Javier Rupérez,[6] Coral
Borja Ibañez,[3,7]† Thomas Langer[1,2,8]†

Mitochondrial morphology is shaped by fusion and division of their me
that adult myocardial function depends on balanced mitochondrial fusio
by processing of the dynamin-like guanosine triphosphatase OPA1 by the
peptidases YME1L and OMA1. Cardiac-specific ablation of Yme1l in mice
accelerated OPA1 proteolysis, which triggered mitochondrial fragmentati
metabolism. This caused dilated cardiomyopathy and heart failure. Cardi
mitochondrial morphology were rescued by Oma1 deletion, which prevent
Feeding mice a high-fat diet or ablating Yme1l in skeletal muscle restored
preserved heart function without suppressing mitochondrial fragmentati
OPA1 is sufficient to maintain heart function, OMA1 is a critical regulator o
and mitochondrial morphology and cardiac metabolism are intimately li

The dynamic behavior of mitochondria pre-
serves mitochondrial integrity and distri-
bution and allows mitochondrial shape
and function to be adapted to altered physio-
logical demands (1, 2). Disturbed mitochon-
drial dynamics is associated with a number of
neurodegenerative disorders and cardiac hyper-
trophy in mice (3, 4). Dynamin-like guanosine
triphosphatases (GTPases) mediate the fusion
and fission of mitochondrial membranes. Mito-
fusins 1 and 2 (MFN1 and MFN2) orchestrate
outer mitochondrial membrane fusion, whereas
OPA1 is required for inner mitochondrial mem-
brane fusion. Fission, on the other hand, is as-
sisted by dynamin-related protein 1 (DRP1), a
cytosolic protein that is recruited to the mito-
chondrial surface in response to various physio-
logical cues. This complex machinery, including
DRP1-specific receptor proteins and cytoskeletal

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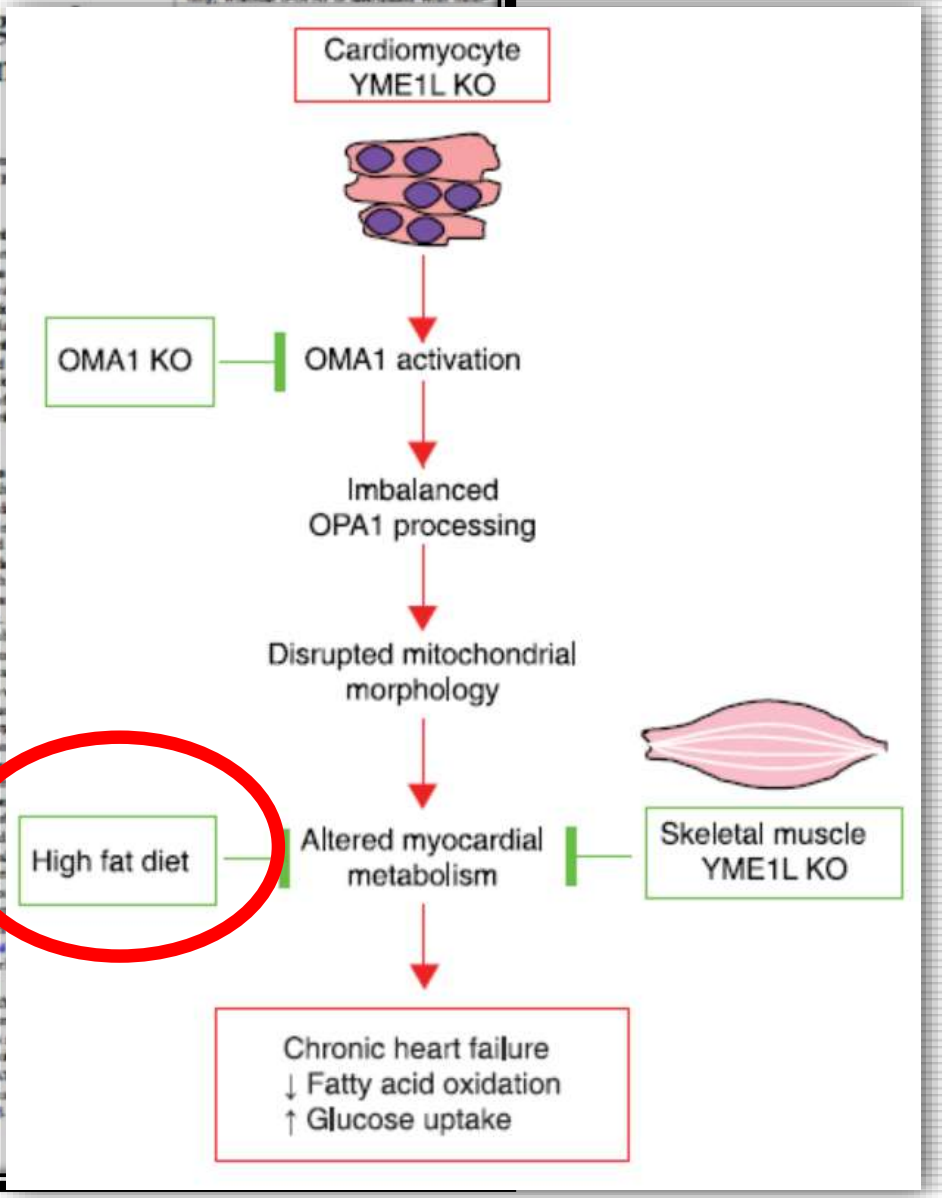
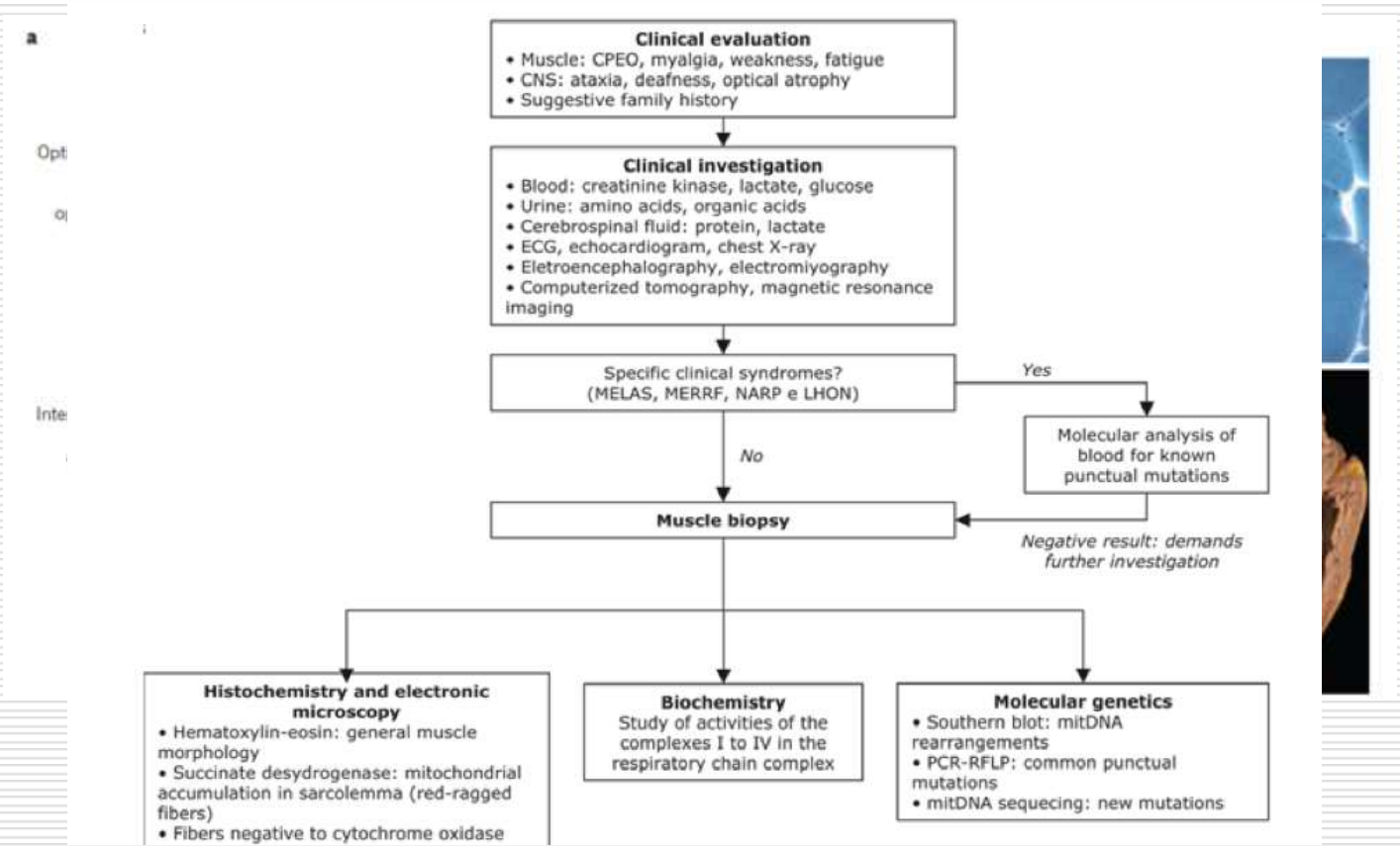
inner membrane, OMA1 and the i-AAA protease
YME1L, convert long OPA1 forms (L-OPA1) into
short forms (S-OPA1) (20–23). The balanced ac-
cumulation of both forms maintains normal mito-
chondrial morphology: Fusion depends on L-OPA1
only, whereas S-OPA1 is associated with mito-



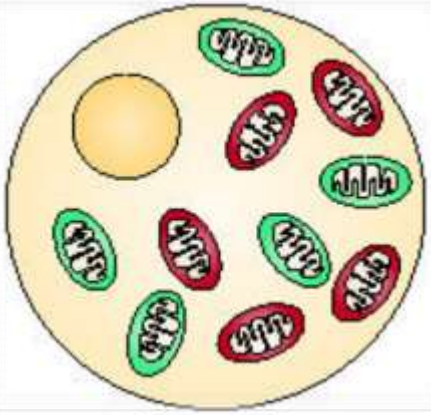
如何诊断线粒体病?


Clinical evaluation
• Muscle: CPEO, myalgia, weakness, fatigue
• CNS: ataxia, deafness, optical atrophy
• Suggestive family history
Clinical investigation
• Blood: creatinine kinase, lactate, glucose
• Urine: amino acids, organic acids
• Cerebrospinal fluid: protein, lactate
• ECG, echocardiogram, chest X-ray
• Eletroencephalography, electromiyography
• Computerized tomography, magnetic resonance imaging
Specific clinical syndromes? (MELAS, MERRF, NARP e LHON)
Yes
No
Molecular analysis of blood for known punctual mutations
Muscle biopsy
Negative result: demands further investigation
Histochemistry and electronic microscopy
• Hematoxylin-eosin: general muscle morphology
• Succinate desydrogenase: mitochondrial accumulation in sarcolemma (red-ragged fibers)
• Fibers negative to cytochrome oxidase
Biochemistry
Study of activities of the complexes I to IV in the respiratory chain complex
Molecular genetics
• Southern blot: mitDNA rearrangements
• PCR-RFLP: common punctual mutations
• mitDNA sequecing: new mutations

如何治疗线粒体病?

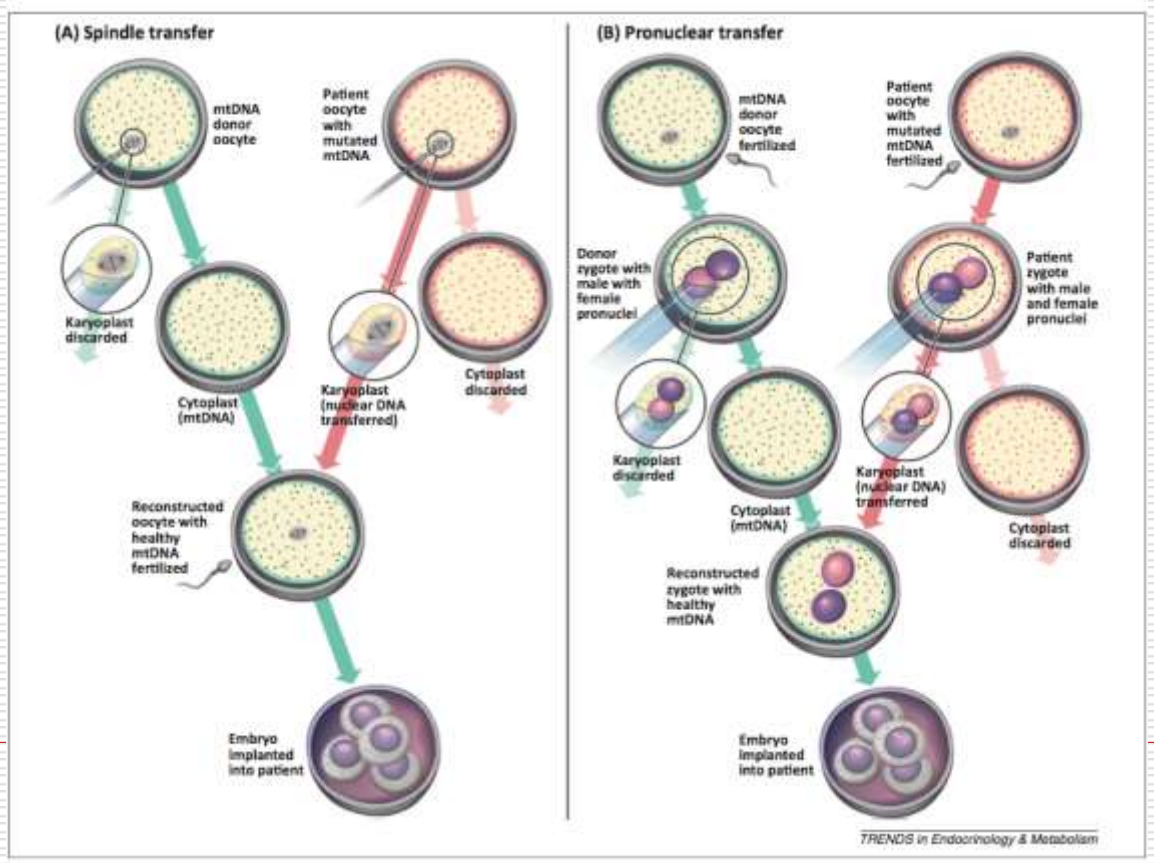
一般处理：规律性体育疗法、充足睡眠、 避免引起线粒体损伤因素等

药物治疗：缓解症状，解决继发性损害



如何治疗线粒体病?

微手术核移植治疗 (Germline gene therapy)：
Spindle transfer (ST) and Pronuclear transfer (PNT)



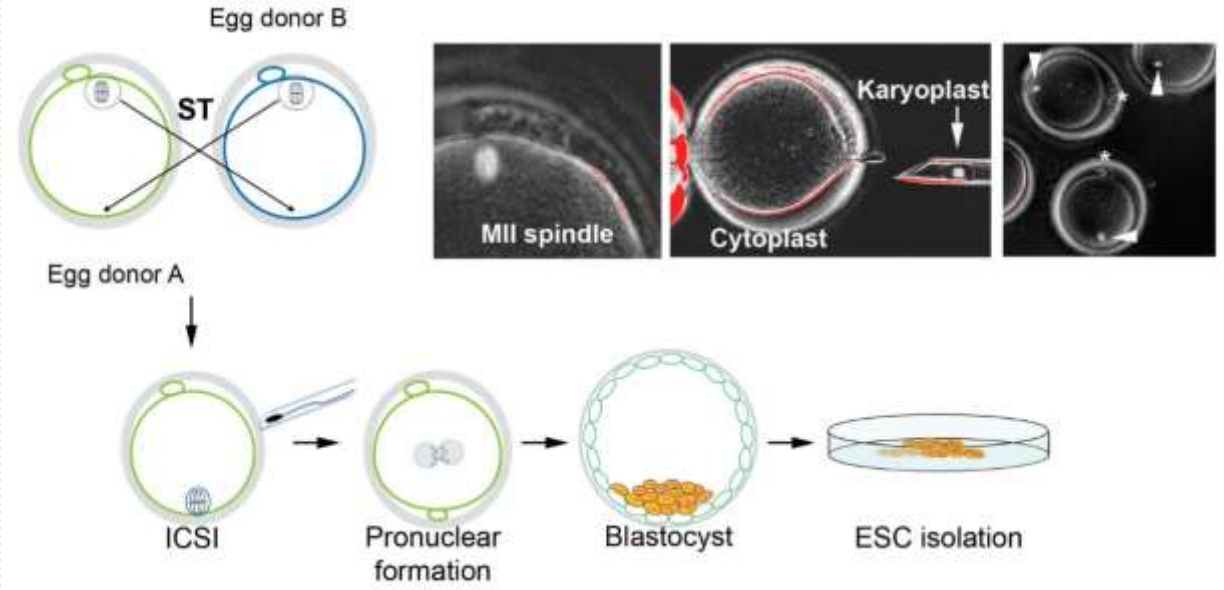
ARTICLE

doi:10.1038/nature11647

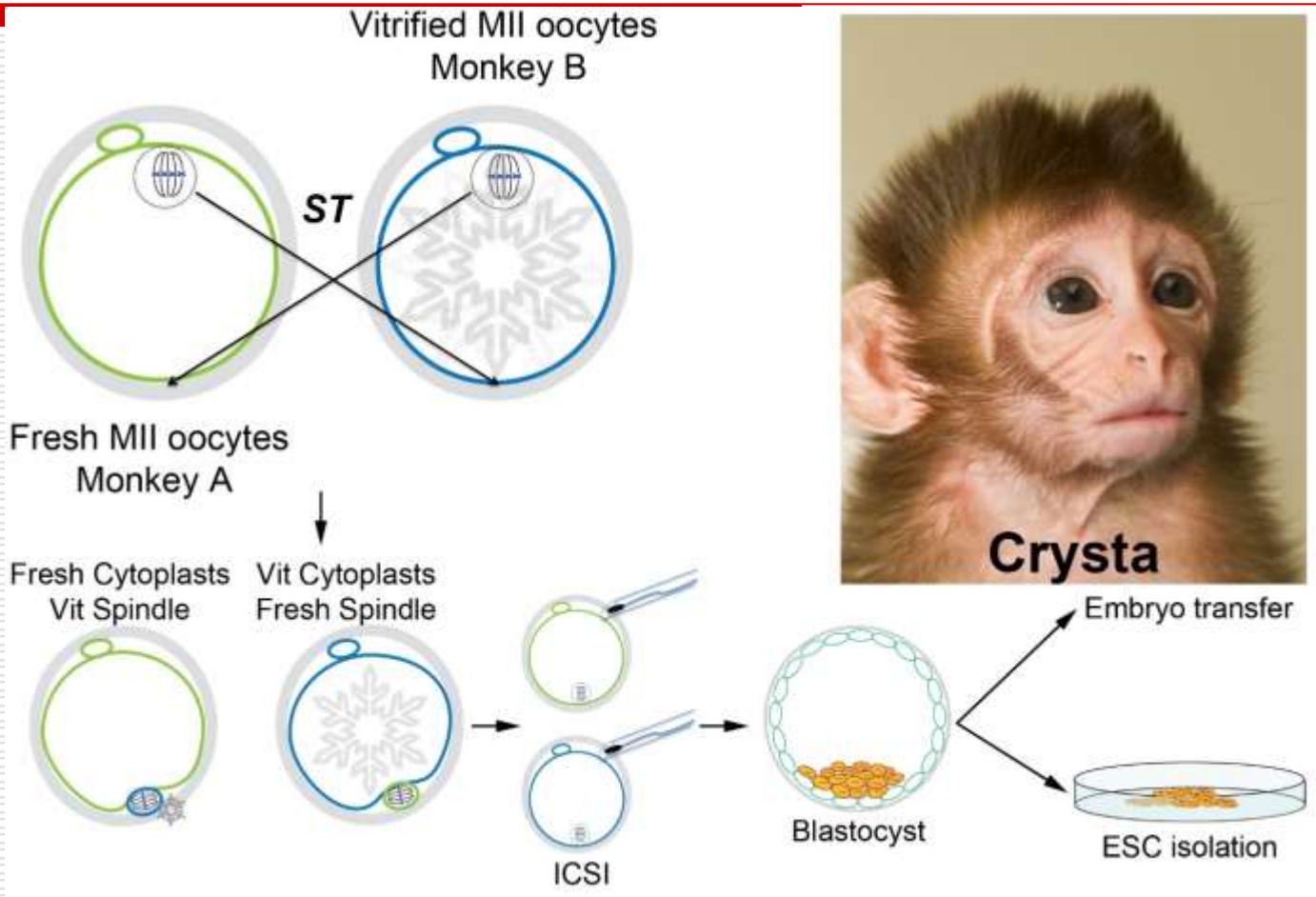
Towards germline gene therapy of inherited mitochondrial diseases

Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2] & Shoukhrat Mitalipov[1,2]

- 7 egg donors
- A total of 106 mature MII oocytes used for ST or served as controls

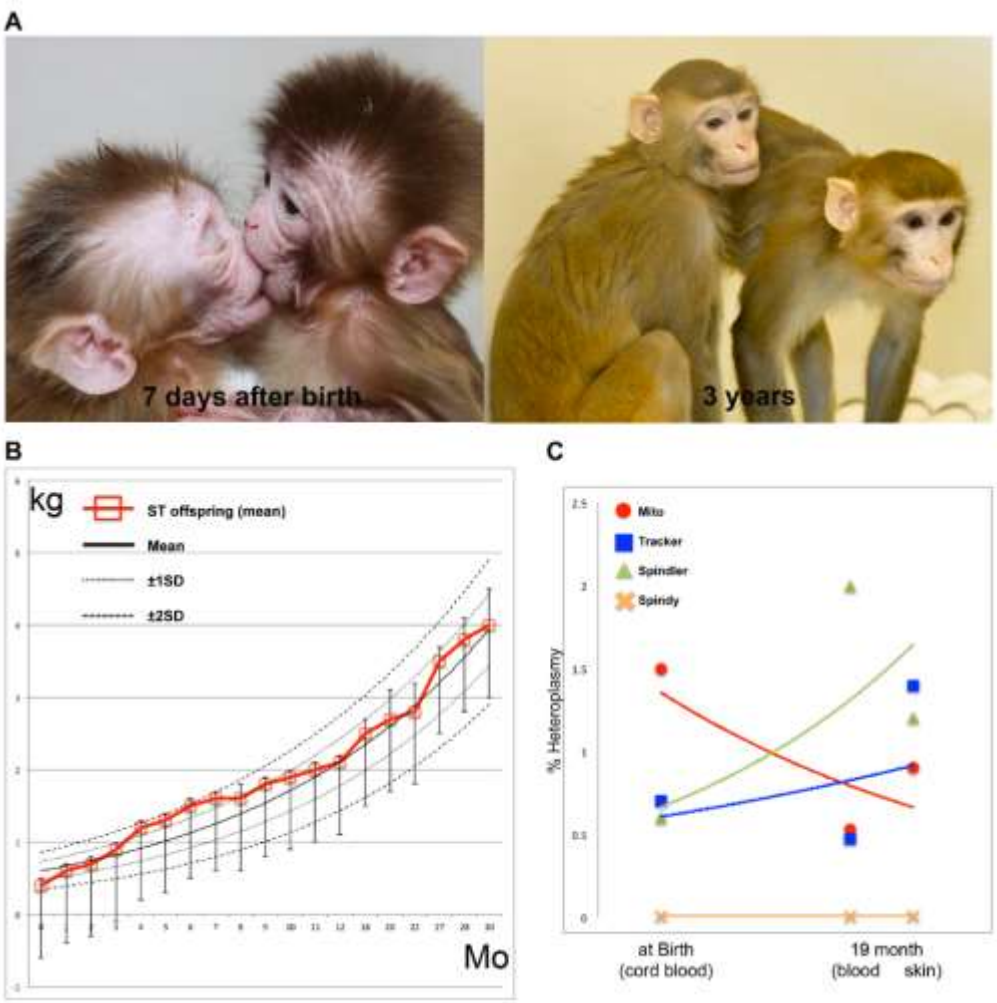


Cryopreservation of oocytes before ST



Tachibana et al., Nature, 2013

Normal growth and development of monkey offspring following mtDNA replacement



Tachibana et al., Nature 2013

线粒体基因缺陷改造新策略（Nature, 2015）

LETTER

doi:10.1038/nature14546

Metabolic rescue in pluripotent cells from patients with mtDNA disease

Hong Ma[1,2], Clifford D. L. Folmes[3], Jun Wu[4], Robert Morey[5], Sergio Mora-Castilla[5], Alejandro Ocampo[4], Li Ma[4], Joanna Poulton[6], Xinjian Wang[7], Riffat Ahmed[1,2], Eunju Kang[1,2], Yeonmi Lee[1,2], Tomonari Hayama[1,2], Ying Li[1,2], Crystal Van Dyken[1,2], Nuria Marti Gutierrez[1,2], Rebecca Tippner-Hedges[1,2], Amy Koski[1,2], Nargiz Mitalipov[1,2], Paula Amato[8], Don P. Wolf[2], Taosheng Huang[7], Andre Terzic[3], Louise C. Laurent[5], Juan Carlos Izpisua Belmonte[4] & Shoukhrat Mitalipov[1,2]

Mitochondria have a major role in energy production via oxidative phosphorylation[1], which is dependent on the expression of critical genes encoded by mitochondrial (mt)DNA. Mutations in mtDNA can cause fatal or severely debilitating disorders with limited treatment options[2]. Clinical manifestations vary based on mutation type and heteroplasmy (that is, the relative levels of mutant and wild-type mtDNA within each cell)[3,4]. Here we generated genetically corrected pluripotent stem cells (PSCs) from patients with mtDNA disease. Multiple induced pluripotent stem (iPS) cell lines were derived from patients with common heteroplasmic mutations including 3243A>G, causing mitochondrial encephalomyopathy and stroke-like episodes (MELAS)[5], and 8993T>G and 13513G>A, implicated in Leigh syndrome. Isogenic MELAS and Leigh syndrome iPS cell lines were generated containing exclusively wild-type or mutant mtDNA through spontaneous segregation of heteroplasmic mtDNA in proliferating fibroblasts. Furthermore, somatic cell nuclear transfer (SCNT) enabled replacement of mutant mtDNA from homoplasmic 8993T>G fibroblasts to generate corrected Leigh-NT1 PSCs. Although Leigh-NT1 PSCs contained donor oocyte wild-type mtDNA (human haplotype D4a) that differed from Leigh syndrome patient haplotype (F1a) at a total of 47 nucleotide sites, Leigh-NT1 cells displayed transcriptomic profiles similar to those in embryo-derived PSCs carrying wild-type mtDNA, indicative of normal nuclear-to-mitochondrial interactions. Moreover, genetically rescued patient PSCs displayed normal metabolic function compared to impaired oxygen consumption and ATP production observed in mutant cells. We conclude that both reprogramming approaches offer complementary strategies for derivation of PSCs containing exclusively wild-type mtDNA, through spontaneous segregation of heteroplasmic mtDNA in individual iPS cell lines or mitochondrial replacement by SCNT in homoplasmic mtDNA-based disease.

and by Leigh syndrome patients carrying heteroplasmic or homoplasmic 8993T>G mutations affecting the ATPase 6 gene (*MT-ATP6*)[3], and heteroplasmic 13513G>A mutation in the *MT-ND5* gene[6]. A panel of ten iPS cell lines from each mutation type was generated and quantitative mtDNA mutation analysis was carried out using amplification refractory mutation system-quantitative polymerase chain reaction (ARMs-qPCR), with a detection threshold of 0.5%. In MELAS iPS cell lines, the mutation was undetectable in five lines and varied from 33% to 100% in the remaining five lines, compared to 29% heteroplasmy in parental fibroblasts (Table 1 and Extended Data Fig. 1a). In iPS cell lines from the heteroplasmic 8993T>G mutation, the mutation was undetectable in one line and ranged from 29% to 87% in the remaining lines, compared to 52% heteroplasmy in parental fibroblasts (Table 1 and Extended Data Fig. 1b). Mutation segregation in individual iPS cell lines from 13513G>A fibroblasts also ranged from 0% to 100%, compared to 84% heteroplasmy in fibroblasts (Table 1 and Extended Data Fig. 1c). Previous studies suggested that segregation of heteroplasmic mtDNA is specific to iPS cells and may occur during or after reprogramming[10,11]. To explore mechanisms, parental fibroblasts carrying 3243A>G and 13513G>A mutations were subcloned and mutation loads in individual clones were analysed. Among ten randomly selected MELAS samples, five were homoplasmic containing either wild type (A) or mutant (G) at the 3243 position. The remaining five contained varying heteroplasmy levels similar to iPS cells (Table 1 and Extended Data Fig. 1d). Variable heteroplasmy levels were also observed in 13513G>A fibroblasts including homoplasmic mutant and wild-type clones (Table 1). Thus, segregation of heteroplasmic mtDNA mutations occurs in skin fibroblasts and may reflect a common phenomenon[12].

Isogenic MELAS iPS cell lines carrying wild-type or mutant mtDNA maintained typical PSC morphology and formed teratomas containing cells and tissues from all three germ layers (Extended Data Fig. 2a, b). We next carried out whole mtDNA sequencing using the

相关专业网站

www.mitomap.org

The United Mitochondrial Disease Foundation—— *www.umdf.org*

谢　谢！