



CHAIRE DE GÉNÉTIQUE ET PHYSIOLOGIE CELLULAIRE

Année universitaire 2013-2014

Pr Christine PETIT



# Le système auditif face à ses agresseurs

06 février 2014

**COURS** : Les agresseurs du système auditif : son, xénobiotiques, vieillissement... Aspects génétiques de la susceptibilité individuelle à ces agresseurs

**SÉMINAIRE** : Prédispositions génétiques aux maladies communes : de la causalité aux facteurs de prédisposition en interaction avec l'environnement  
Jean-Louis Mandel, *IGBMC, université Louis Pasteur, Strasbourg*

06 mars 2014

**COURS** : Le métabolisme de l'oxygène et la toxicité des espèces oxygénées activées, plaque tournante de l'action de nombreux agresseurs

**SÉMINAIRE** : Détection hors fréquence et réponses cochléaires fantômes  
Paul Avan, *laboratoire de biophysique sensorielle, université d'Auvergne, Clermont-Ferrand*

13 mars 2014

**COURS** : Détecteurs et effecteurs du stress oxydant : rôles dans le métabolisme et la signalisation. Le dialogue des organelles : la part des peroxysomes.

**SÉMINAIRE** : Les antibiotiques sont-ils autodestructeurs ?  
Patrice Courvalin, *unité des agents antibactériens, institut Pasteur, Paris*

20 mars 2014

**COURS** : Les moyens de défense : prévention et traitement

**SÉMINAIRE** : Acouphènes subjectifs : physiopathologie et éléments d'une prise en charge rationnelle  
Alain Londero, *Service ORL et CCF, hôpital Georges Pompidou, Paris*

1<sup>er</sup> cours : 6 février 2014

**Les agresseurs du système auditif : son, xénobiotiques, vieillissement...**

**Aspects génétiques de la susceptibilité individuelle à ces agresseurs.**

Séminaire:

**Jean-Louis Mandel** – IGBMC, université Louis Pasteur, Strasbourg  
**“Prédispositions génétiques aux maladies communes : de la causalité aux facteurs de prédisposition en interaction avec l'environnement”**

## **Les agresseurs du système auditif : son, xénobiotiques, vieillissement...**

### **Aspects génétiques de la susceptibilité individuelle à ces agresseurs.**

1. Les agents agresseurs
2. La susceptibilité génétique à ces agents et la pathogénie des atteintes auditives associées.
  - a. le cisplatine
  - b. les aminoglycosides
3. Les infrasons: agents agresseurs des systèmes auditif et vestibulaire?

# **Les agresseurs du système auditif : son, xénobiotiques, vieillissement...**

## **Aspects génétiques de la susceptibilité individuelle à ces agresseurs.**

### **1. Les agents agresseurs**

# LE SON

*caractérisé par :*

**sa hauteur** (grave ou aigu): fréquence de l'onde de pression

- mesure du nombre de cycles de compression-décompression par seconde

$$1 \text{ Hz} = 1 \text{ cycle/sec } (\lambda = 344 \text{ m})$$

$$1000 \text{ Hz} = 1000 \text{ cycles/sec } (\lambda = 34 \text{ cm})$$

$$442 \text{ Hz} = \text{la du diapason (la}_3 \text{ du piano)}$$

**son intensité:** amplitude de l'onde de pression

- mesure en dB:

$$\begin{aligned} \text{dBSPL} &= 10 \log_{10}(p/p_r)^2 = 20 \log_{10}(p/p_r) \quad (\text{N/m}^2) \quad 1 \text{ N/m}^2 = 1 \text{ Pa} \\ &= 10 \log_{10}(I/I_r) \quad (\text{watt/m}^2) \end{aligned}$$

$$p_r = 20 \mu \text{ Pa} = \text{pression minimale perceptible (homme)}$$

une variation de 3dB= correspond à un doublement de l'intensité sonore de référence

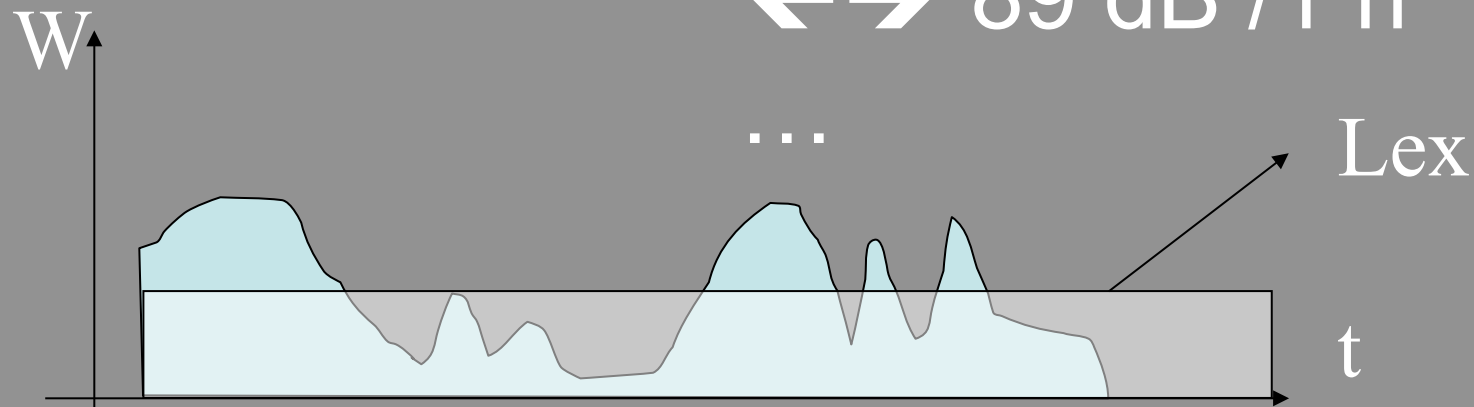
# Iso-énergie

norme : énergie < 80 dB (A) – 8 h / jour de travail

80 dB / 8 h ↔ 83 dB / 4 h

↔ 86 dB / 2 h

↔ 89 dB / 1 h

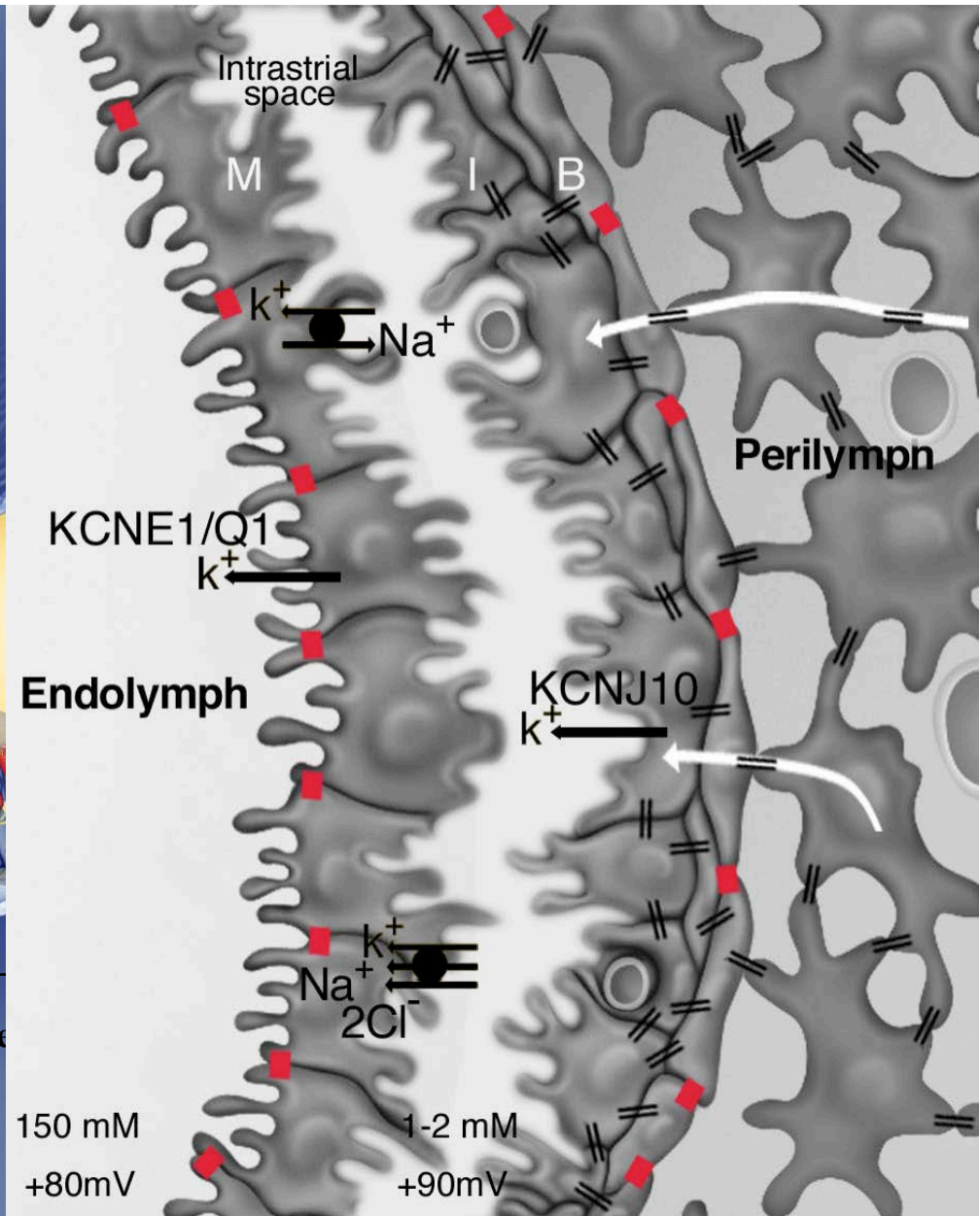
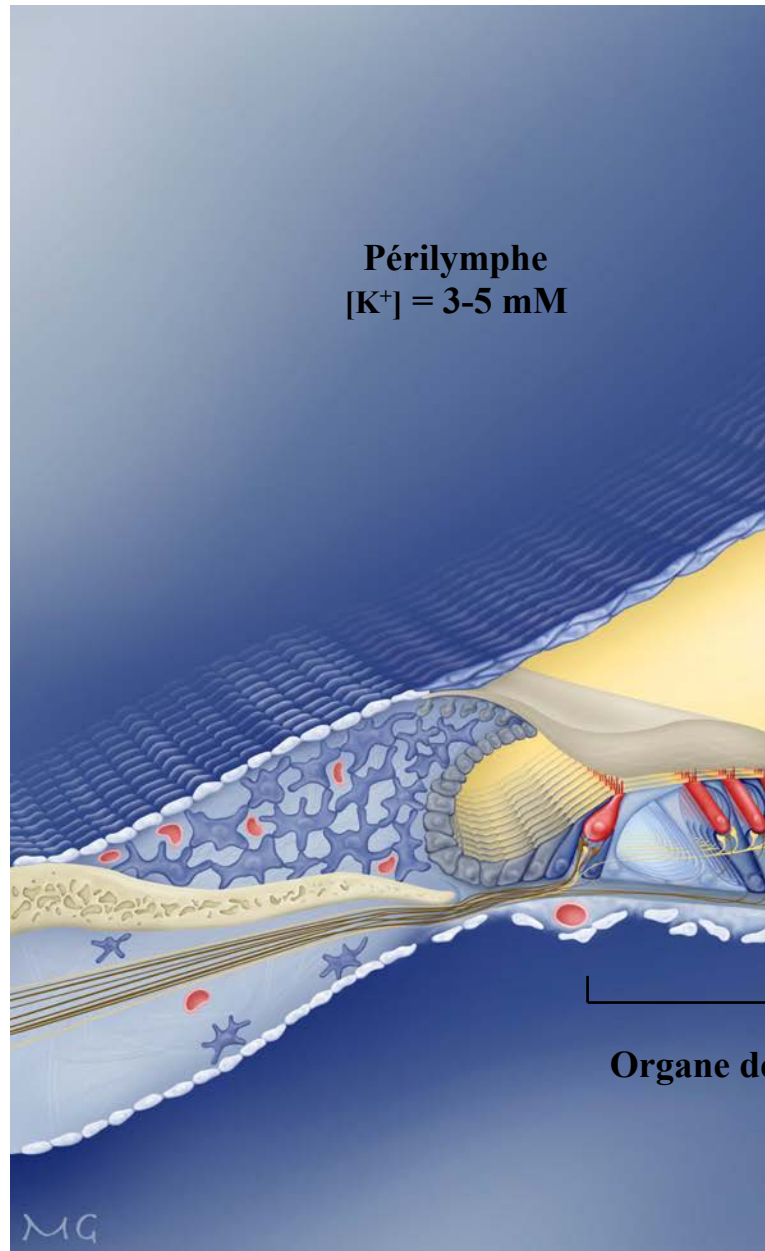


Paul Avan

# Ototoxicité médicamenteuse

- Plus de 130 médicaments répertoriés comme étant ototoxiques.
- Cet effet est généralement dépendant de la dose, parfois du mode d'administration.
- Le plus souvent l'atteinte est réversible, son mécanisme inconnu et la susceptibilité à la toxicité très différente d'un individu à l'autre.
- Quelques exemples:
  - **Les salicylés et autres anti-inflammatoires non stéroïdiens:** l'acide acétylsalicylique (aspirine), diclofenac, ibuprofène, indométhacine, kétoprofène, piroxicam, phenylbutazone.  
l'acide acétylsalicylique inhibe l'électromotilité (atteinte de la fonction d'amplification) des cellules ciliées externes CCEs
  - **Certains antipaludéens:** Quinine et chloroquine; le plus souvent réversible; affecte l'électromotilité des CCEs et bloque le canal de mécanotransduction auditive (quinine)
  - **Certains diurétiques:**
    - ouabaine; peut être irréversible; inhibe l'ATPase  $\text{Na}^+, \text{K}^+$  de la strie vasculaire;
    - diurétiques de l'anse du tubule rénal, furosémide et bumétamide: agissent sur le cotransporteur  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}$  de la strie vasculaire; ototoxicité par voie veineuse, le plus souvent réversible.
  - **Certains antibiotiques:**
    - aminoglycosides: streptomycine, néomycine, gentamycine, kanamycine, amikacine, sisomycine, tobramycine, netilmycine, dihydrostreptomycine...; surdité bilatérale, symétrique et irréversible (unilatérale parfois: kanamycine);
    - érythromycine; surdité le plus souvent réversible; origine strie vasculaire ou système auditif central?
    - vancomycine; irréversible.
  - **Certains antinéoplasiques** (chimiothérapie): cisplatine, vincristine, moutardes azotées, vinblastine, carboplastine, bleomycine, cisplatine, atteinte irréversible.







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## Les drogues illicites ...

opiacés; opioïdes

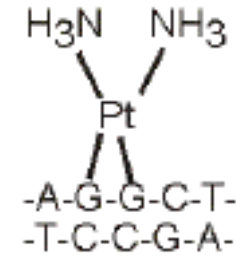
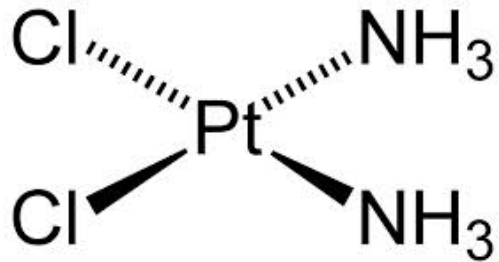
hydrocodone/acétaminophène (paracétamol)

## **Les agresseurs du système auditif : son, xénobiotiques, vieillesse...**

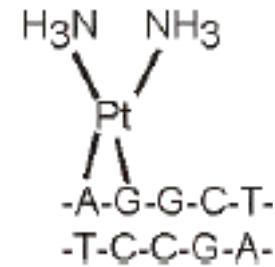
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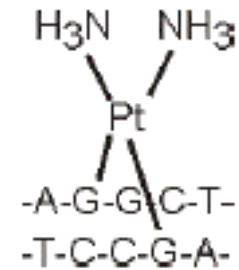
# Nature des adduits bifonctionnels du cisplatine formés avec l'ADN



d(GpG)

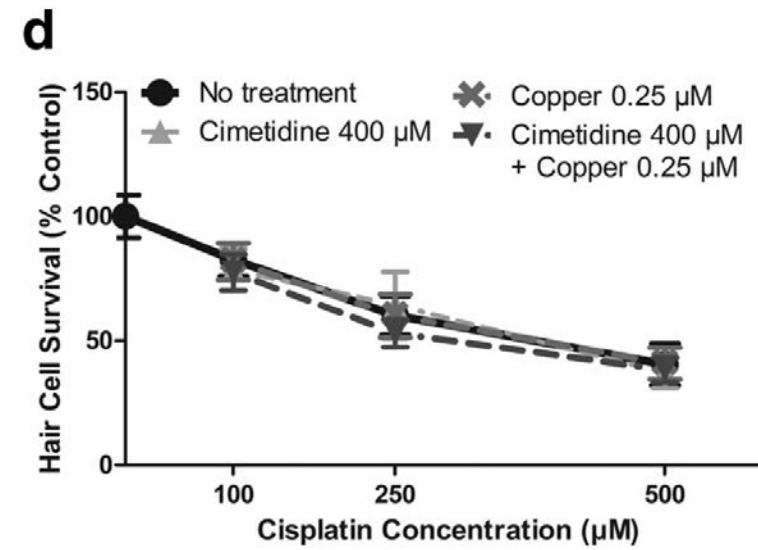
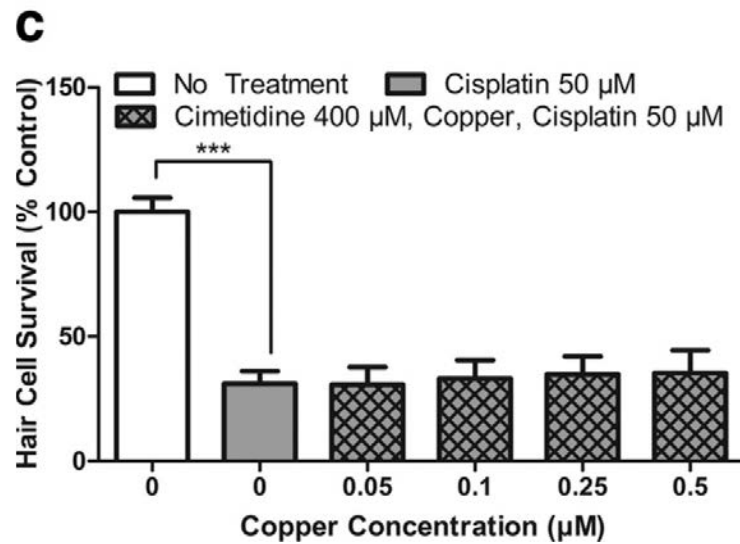
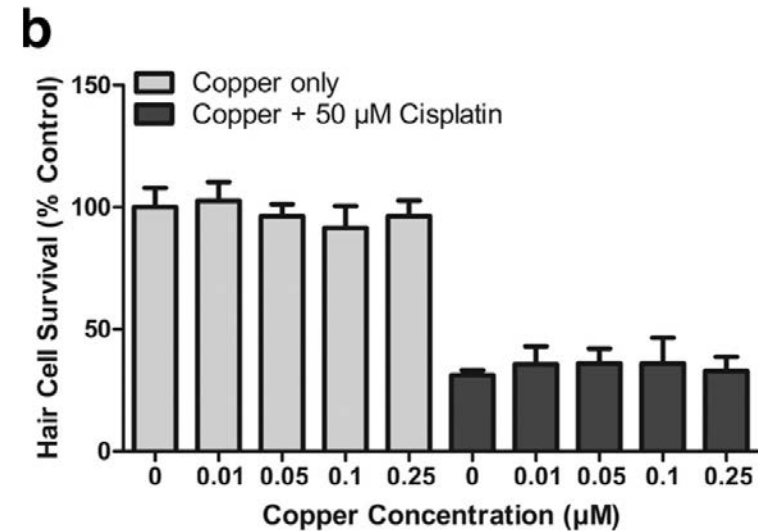
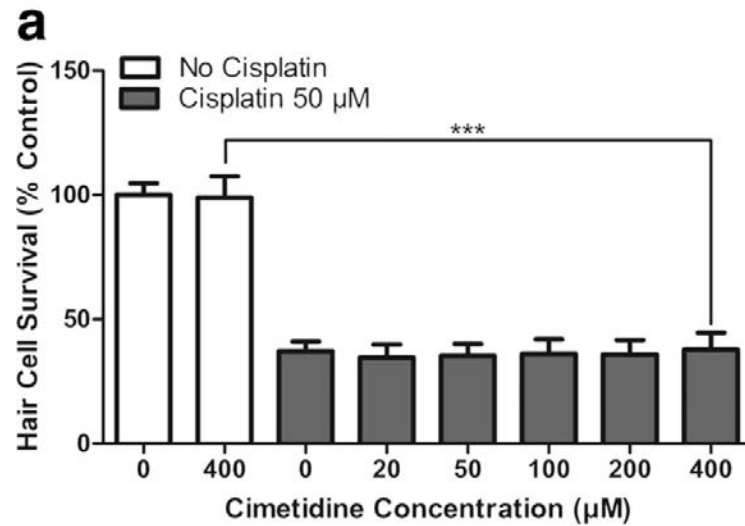


d(ApG)

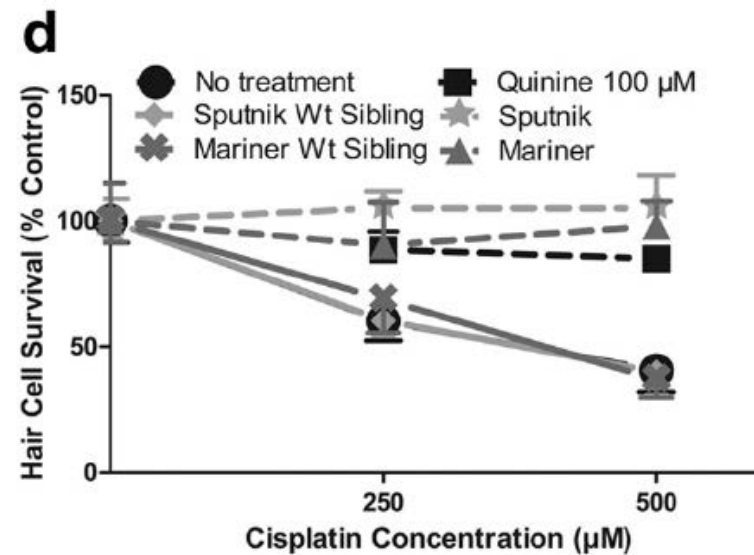
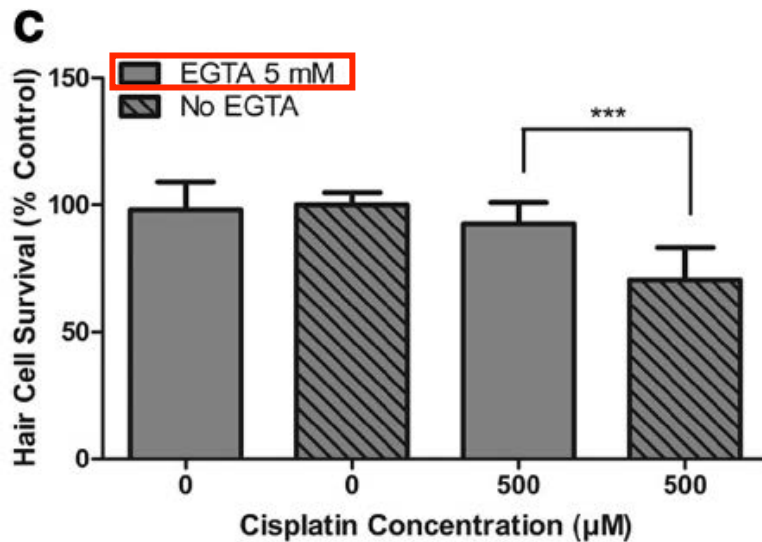
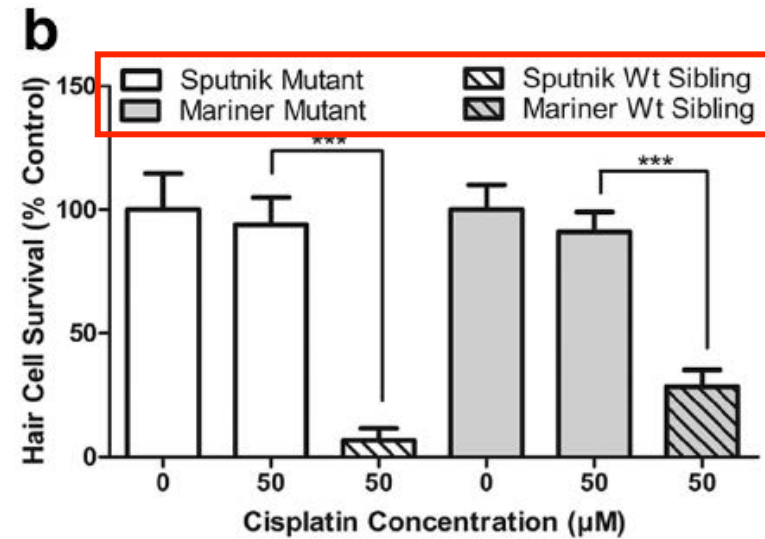
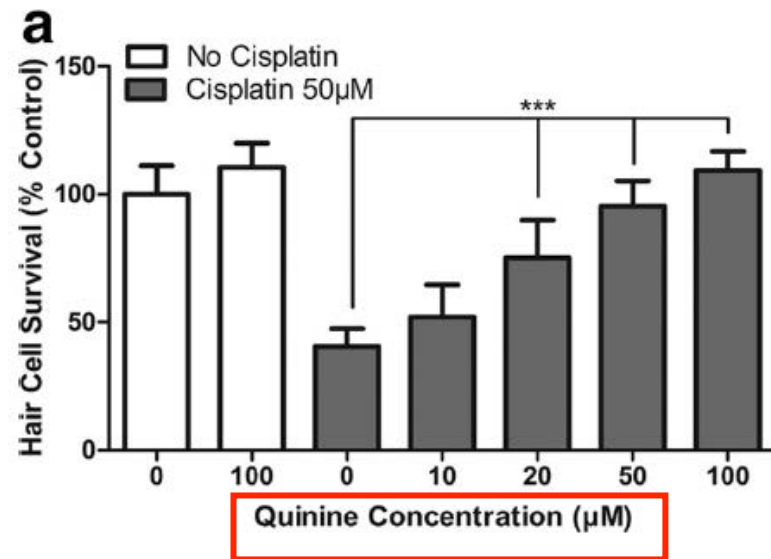


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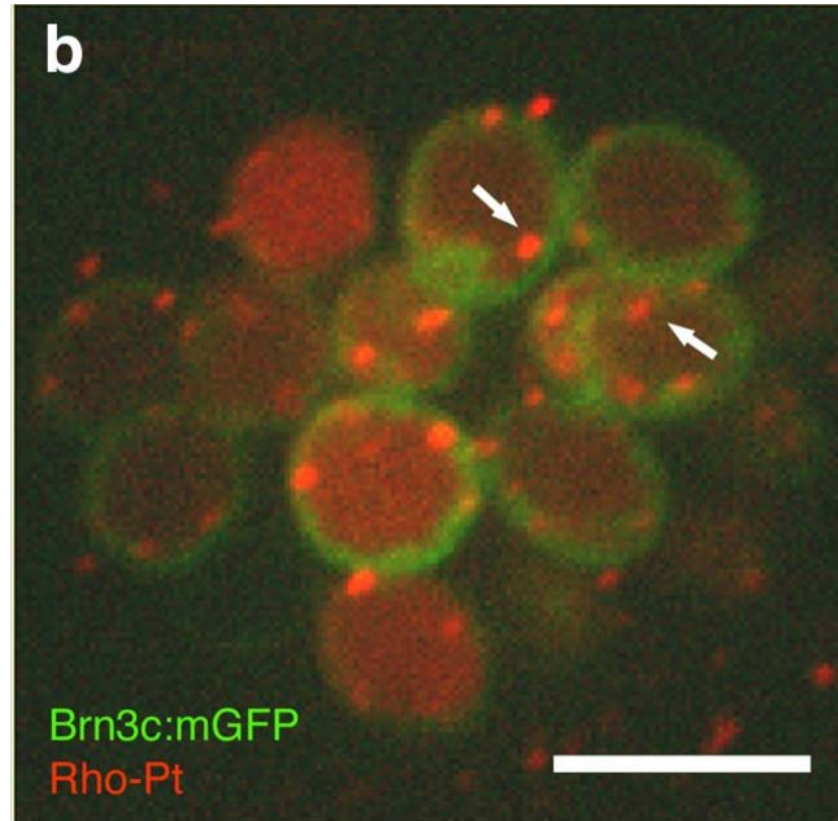
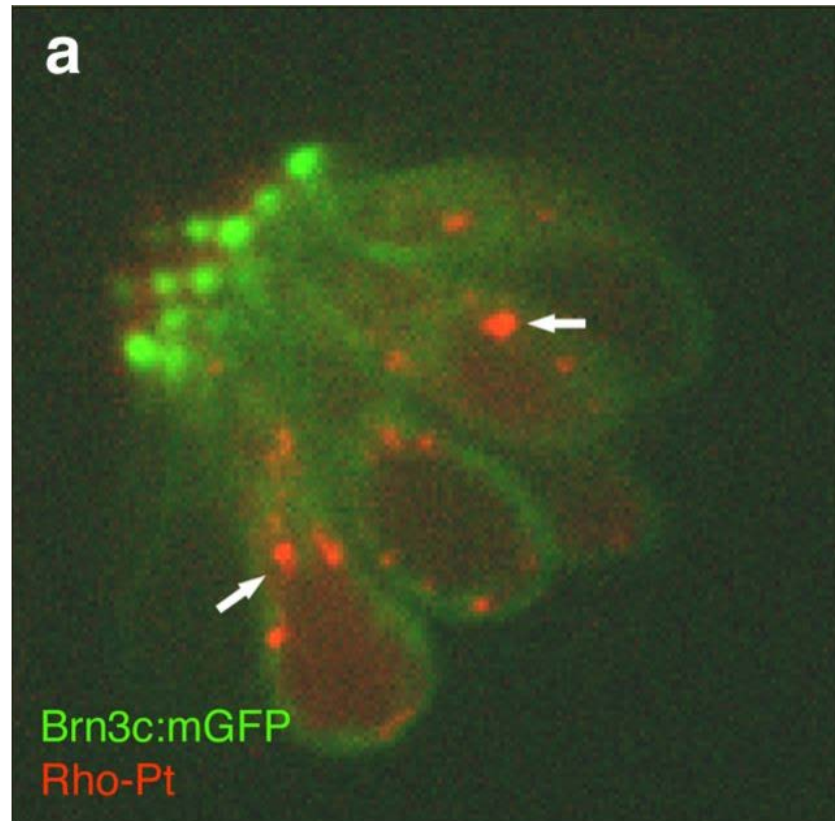
# Low-concentration copper and/or cimetidine treatment does not prevent cisplatin-induced hair cell death



# Functional mechanotransduction is required for cisplatin-induced hair cell death in the zebrafish lateral line



# Rho-Pt uptake in lateral line hair cells

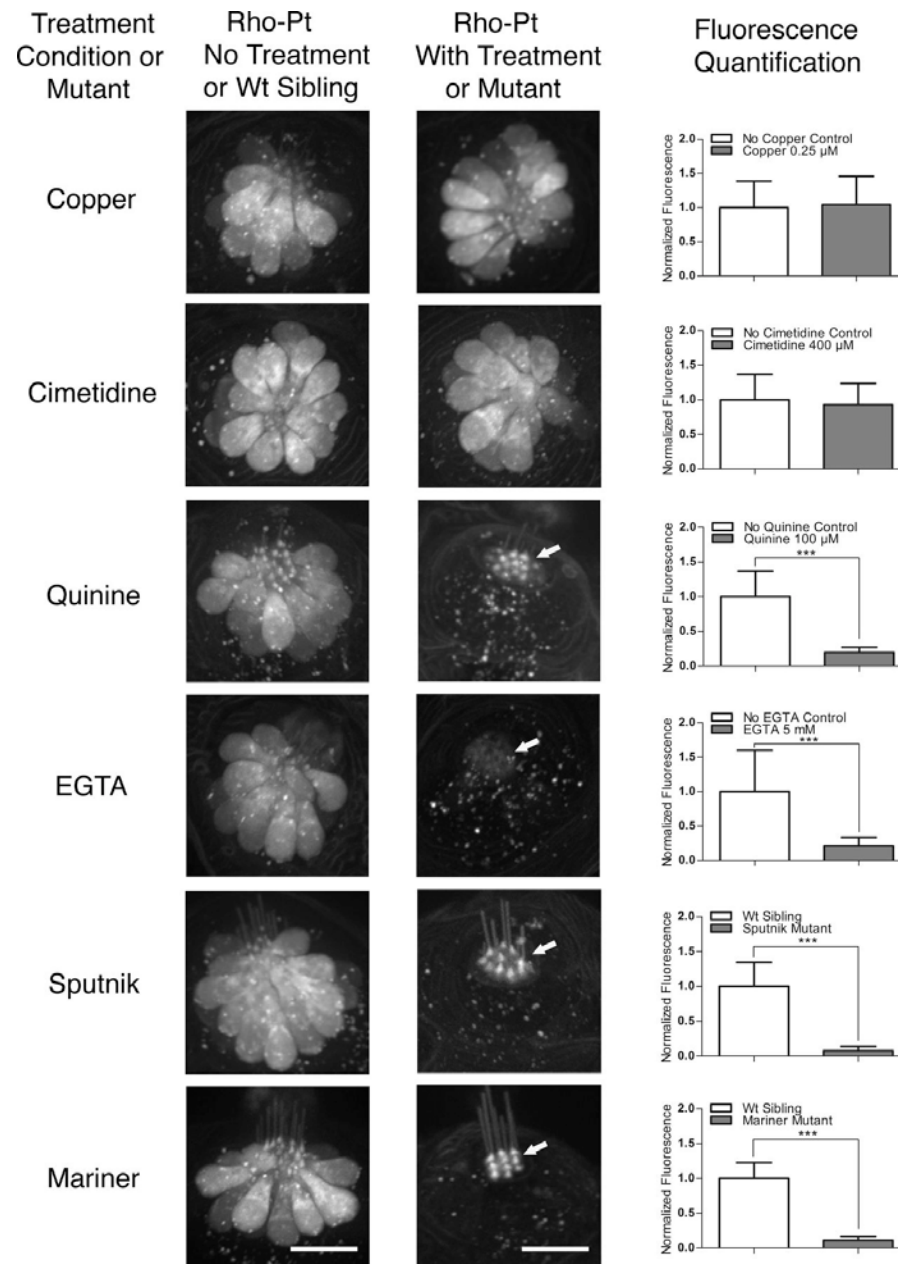




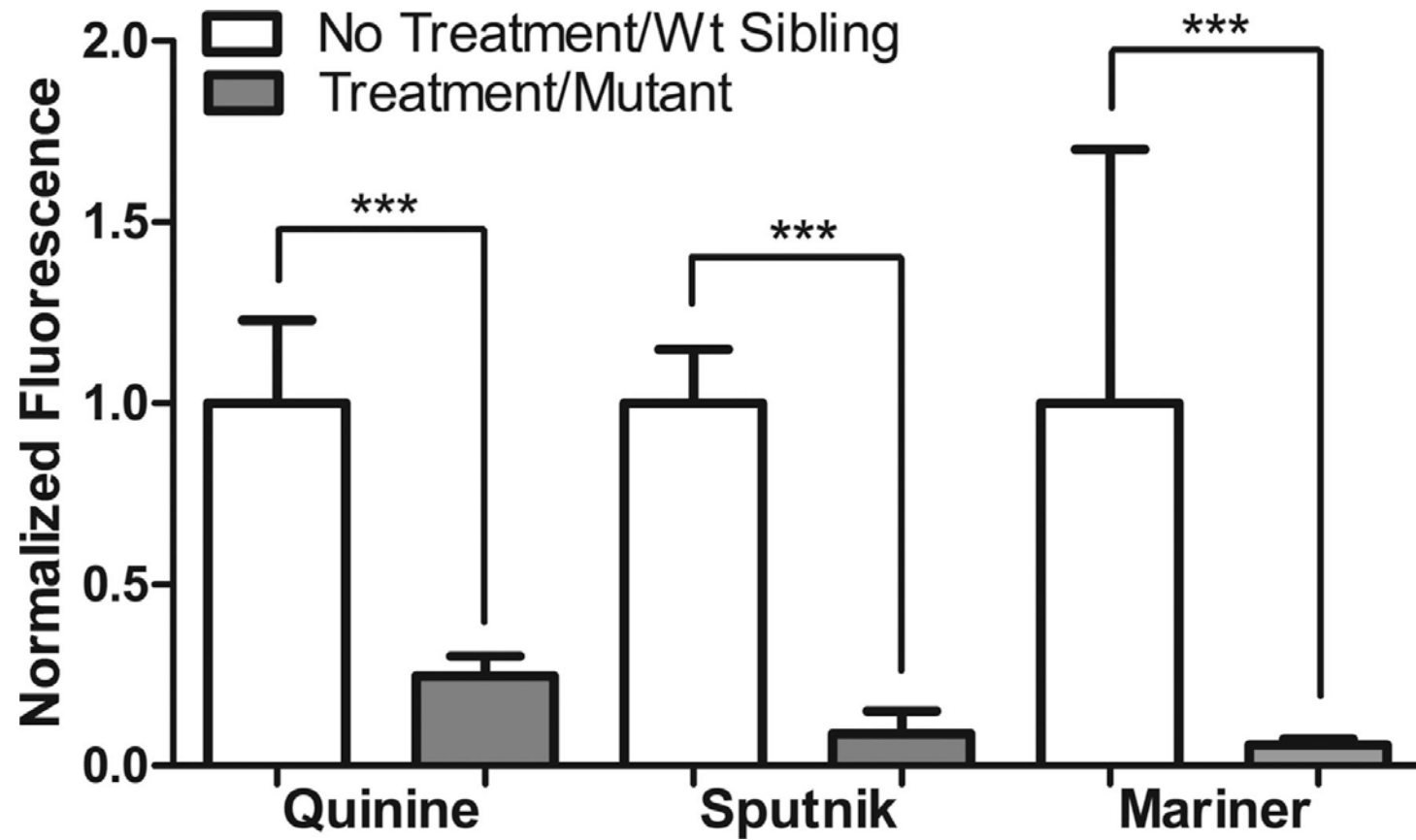
# Functional mechanotransduction is required for uptake of Rho-Pt.

## Chemical or genetic inhibition of mechanotransduction leads to reduction in Rho-Pt uptake

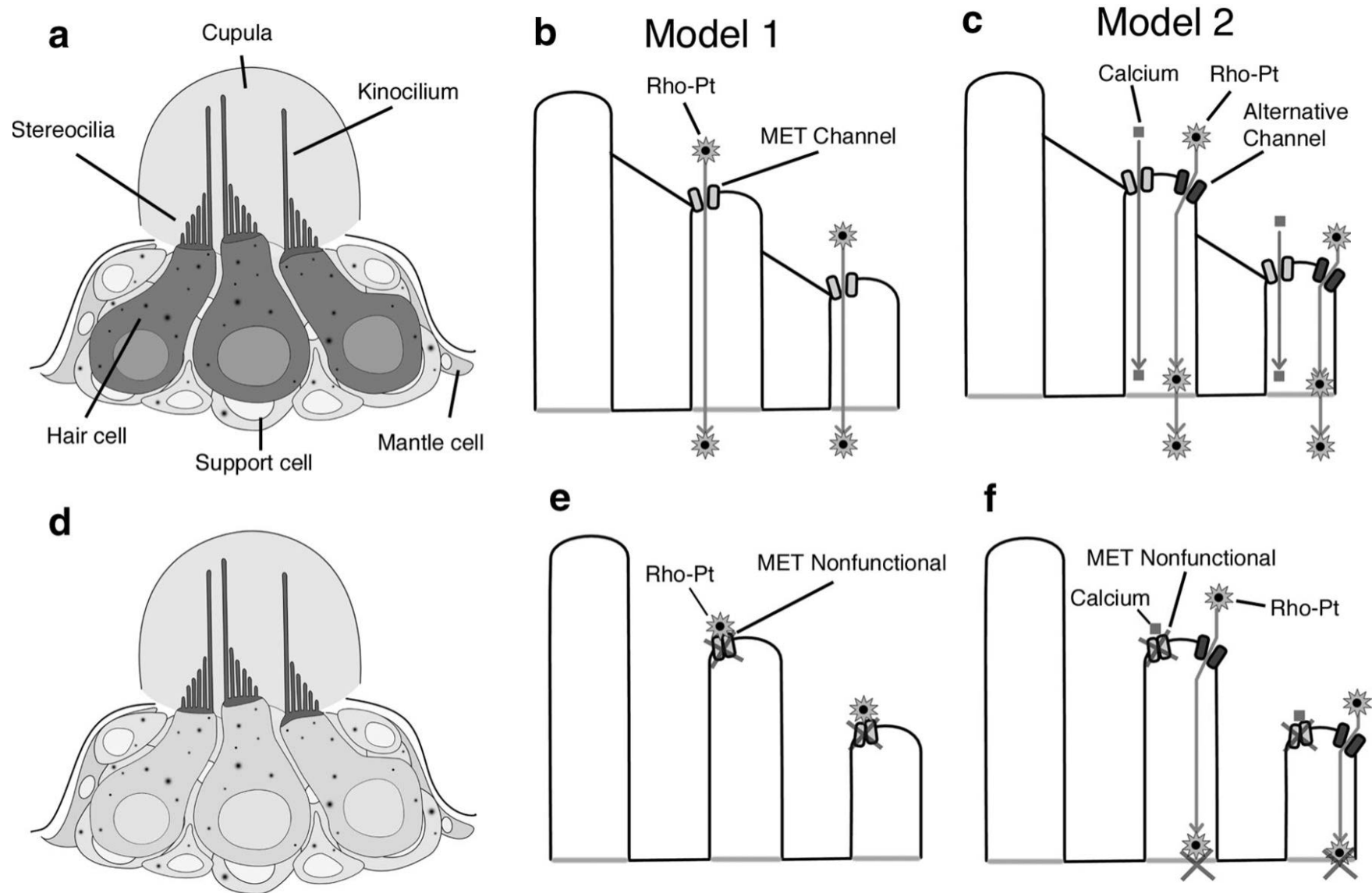
Thomas AJ... Ou HC *J Neurosci* 33, 2013



# Rho-Pt uptake remains inhibited after 4 h continuous exposure



# Two models of cisplatin entry into hair cells



# Genetic variants associated with cisplatin-induced hearing loss

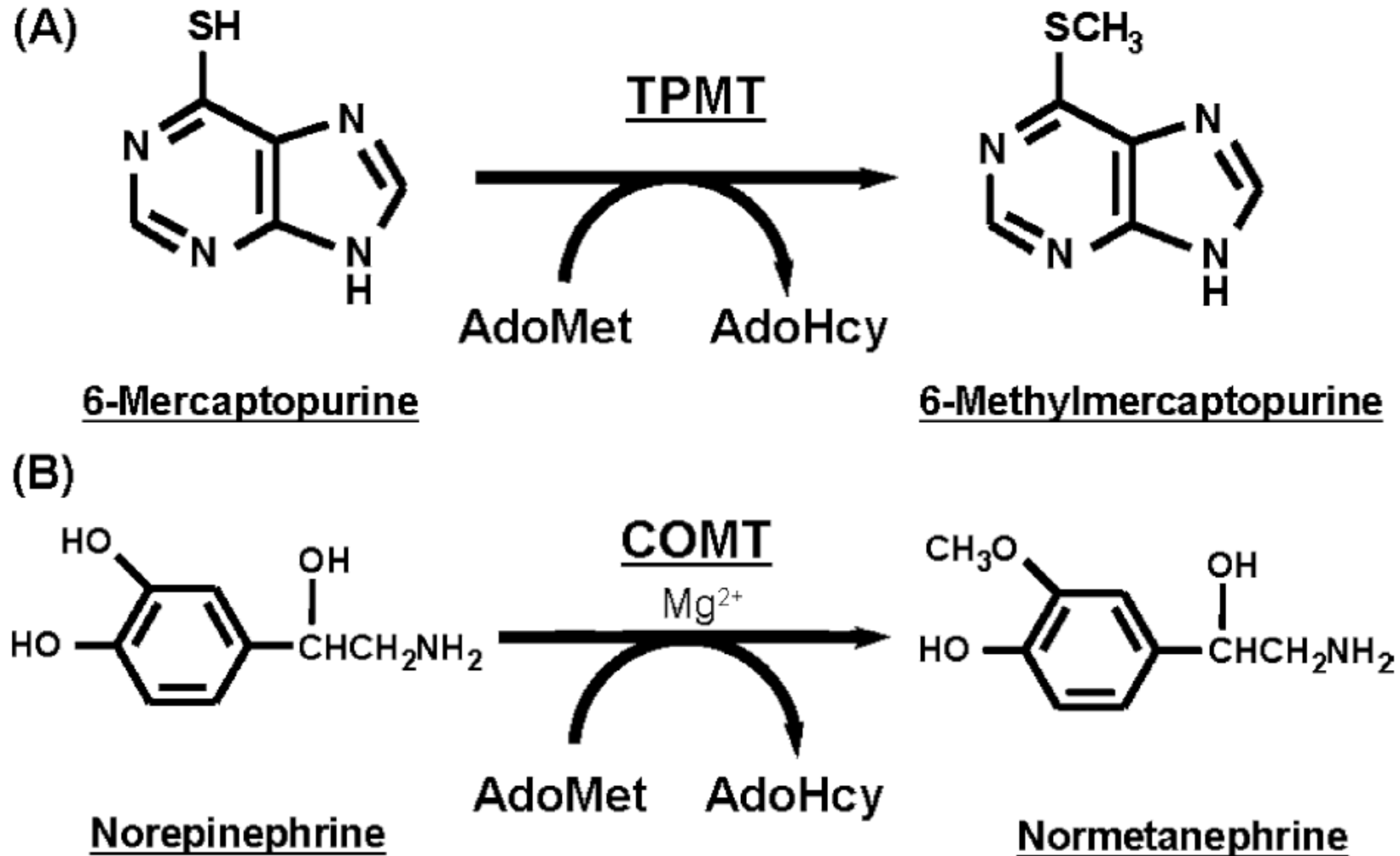
SNP	Genotype/ allele	Discovery (n=53)			Replication (n = 109)			Combined (n = 162)			Adjusted P value <sup>b</sup>			
		Ototox. (n = 33)	Controls (n = 20)	OR (95% CI)	P value <sup>a</sup>	Ototox. (n = 73)	Controls (n = 36)	OR (95% CI)	P value <sup>a</sup>	Ototox. (n = 106)		Controls (n = 56)	OR (95% CI)	P value <sup>a</sup>
<b>TPMT</b>														
rs12201199	A/A	1	0	2.51 (0.1, 65.00)	–	2	0	3.09 (0.14, 66.17)	0.528	3	0	4.77 (0.24, 94.11)	0.277	1.000
	A/T	8	0	14.22 (0.77, 261.62)	0.017	14	1	8.60 (1.08, 68.26)	0.018	22	1	14.94 (1.96, 114.09)	0.00061	0.086
	A/_	9	0	15.90 (0.87, 290.02)	0.0097	16	1	9.82 (1.25, 77.37)	0.010	25	1	16.98 (2.23, 128.99)	<b>0.000181<sup>c</sup></b>	<b>0.0231<sup>c</sup></b>
	T/T	24	20	1		57	35	1		81	55	1		
	A	10	0	14.29 (0.81, 251.74)	0.0097	18	1	9.98 (1.31, 76.36)	0.0071	28	1	16.89 (2.27, 125.88)	<b>0.00022<sup>c</sup></b>	<b>0.0318<sup>c</sup></b>
	T	56	40			128	71			184	111			
rs1142345	G/G	0	0	–	1	2	0	2.75 (0.13, 58.92)	0.543	2	0	3.10 (0.15, 65.79)	0.527	1.000
	G/A	8	0	13.67 (0.74, 251.10)	0.019	7	1	3.83 (0.45, 32.39)	0.264	15	1	9.27 (1.19, 72.15)	0.011	0.812
	G/_	8	0	13.67 (0.74, 251.10)	0.019	9	1	4.92 (0.60, 40.46)	0.160	17	1	10.51 (1.36, 81.17)	<b>0.0068</b>	0.633
	A/A	25	20	1		64	35	1		89	55	1		
	G	8	0	11.03 (0.61, 197.64)	0.022	11	1	5.79 (0.73, 45.72)	0.044	19	1	10.93 (1.44, 82.74)	<b>0.0017</b>	0.221
	A	58	40			135	71			193	111			
rs1800460	A/A	0	0	–	1	1	0	1.60 (0.06, 40.34)	1.000	1	0	1.82 (0.07, 45.45)	0.999	1.000
	A/G	8	0	13.67 (0.74, 251.10)	0.019	6	0	6.94 (0.38, 126.78)	0.174	14	0	17.59 (1.03, 300.74)	0.0020	0.244
	A/_	8	0	13.67 (0.74, 251.10)	0.019	7	0	8.01 (0.44, 144.31)	0.094	15	0	18.80 (1.10, 320.51)	<b>0.0013</b>	0.175
	G/G	25	20	1		66	35	1		91	55	1		
	A	8	0	11.03 (0.61, 197.64)	0.022	8	0	8.12 (0.46, 143.37)	0.046	16	0	17.96 (1.07, 302.66)	<b>0.00312</b>	0.413
	G	58	40			138	70			196	110			
<b>COMT</b>														
rs4646316	G/G	20	10	21.48 (1.08, 426.70)	0.0093	51	15	6.80 (0.58, 80.28)	0.148	71	25	19.88 (2.33, 169.70)	<b>0.00098</b>	0.135
	G/A	13	5	27.00 (1.27, 575.95)	0.0075	21	19	2.21 (0.19, 26.38)	0.607	34	24	9.92 (1.14, 85.95)	0.022	0.959
	G/_	33	15	23.77 (1.24, 457.45)	0.0054	72	34	4.24 (0.37, 48.34)	0.253	105	49	15.00 (1.80, 125.29)	0.0026	0.321
	A/A	0	5	1		1	2	1		1	7	1		
	G	53	25	2.45 (1.01, 5.91)	0.044	123	49	2.51 (1.29, 4.89)	0.0059	176	74	2.51 (1.48, 4.27)	<b>0.00055</b>	0.076
	A	13	15			23	23			36	38			
rs9332377	A/A	1	0	2.71 (0.10, 70.65)	1.000	4	0	5.59 (0.29, 107.16)	0.293	5	0	7.65 (0.41, 141.32)	0.156	1.000
	A/G	12	2	5.40 (1.06, 27.47)	0.052	14	2	4.71 (1.01, 21.95)	0.051	26	4	4.51 (1.48, 13.68)	0.0052	0.538
	A/_	13	2	5.85 (1.16, 29.54)	0.028	18	2	5.56 (1.21, 25.49)	0.017	31	4	5.37 (1.79, 16.14)	<b>0.00109</b>	0.148
	G/G	20	18	1		55	34	1		75	52	1		
	A	14	2	5.12 (1.10, 23.85)	0.024	22	2	6.21 (1.42, 27.19)	0.0087	36	4	5.52 (1.91, 15.95)	<b>0.000182<sup>c</sup></b>	<b>0.0261<sup>c</sup></b>
	G	52	38			124	70			176	108			

<sup>a</sup>Determined using Fisher exact test. <sup>b</sup>Determined using Bonferroni-corrected P value. <sup>c</sup>P < 0.05 after Bonferroni multiple testing correction. Ototox., individuals with ototoxicity.

COMT catechol O-methyltransferase

TPMT thiopurine S-methyltransferase

# TPMT and COMT-catalyzed Methylation



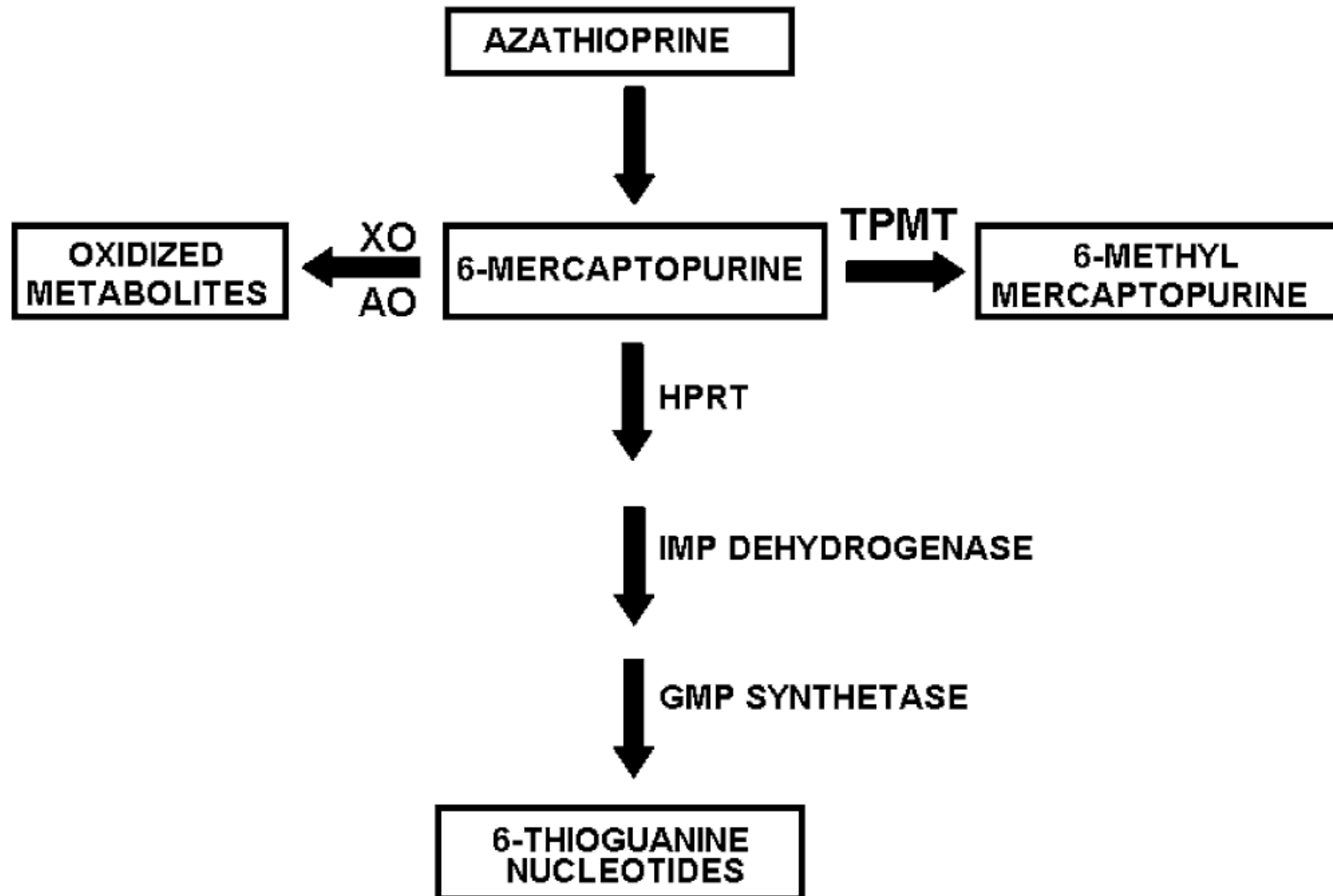
# Genetic variants associated with cisplatin-induced hearing loss

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	G/A	13	5	27.00 (1.27, 575.95)	0.0075	21	19	2.21 (0.19, 26.38)	0.607	34	24	9.92 (1.14, 85.95)	0.022	0.959
	G/_	33	15	23.77 (1.24, 457.45)	0.0054	72	34	4.24 (0.37, 48.34)	0.253	105	49	15.00 (1.80, 125.29)	0.0026	0.321
	A/A	0	5	1		1	2	1		1	7	1		
	G	53	25	2.45 (1.01, 5.91)	0.044	123	49	2.51 (1.29, 4.89)	0.0059	176	74	2.51 (1.48, 4.27)	<b>0.00055</b>	0.076
	A	13	15			23	23			36	38			
rs9332377	A/A	1	0	2.71 (0.10, 70.65)	1.000	4	0	5.59 (0.29, 107.16)	0.293	5	0	7.65 (0.41, 141.32)	0.156	1.000
	A/G	12	2	5.40 (1.06, 27.47)	0.052	14	2	4.71 (1.01, 21.95)	0.051	26	4	4.51 (1.48, 13.68)	0.0052	0.538
	A/_	13	2	5.85 (1.16, 29.54)	0.028	18	2	5.56 (1.21, 25.49)	0.017	31	4	5.37 (1.79, 16.14)	<b>0.00109</b>	0.148
	G/G	20	18	1		55	34	1		75	52	1		
	A	14	2	5.12 (1.10, 23.85)	0.024	22	2	6.21 (1.42, 27.19)	0.0087	36	4	5.52 (1.91, 15.95)	<b>0.000182<sup>c</sup></b>	<b>0.0261<sup>c</sup></b>
	G	52	38			124	70			176	108			

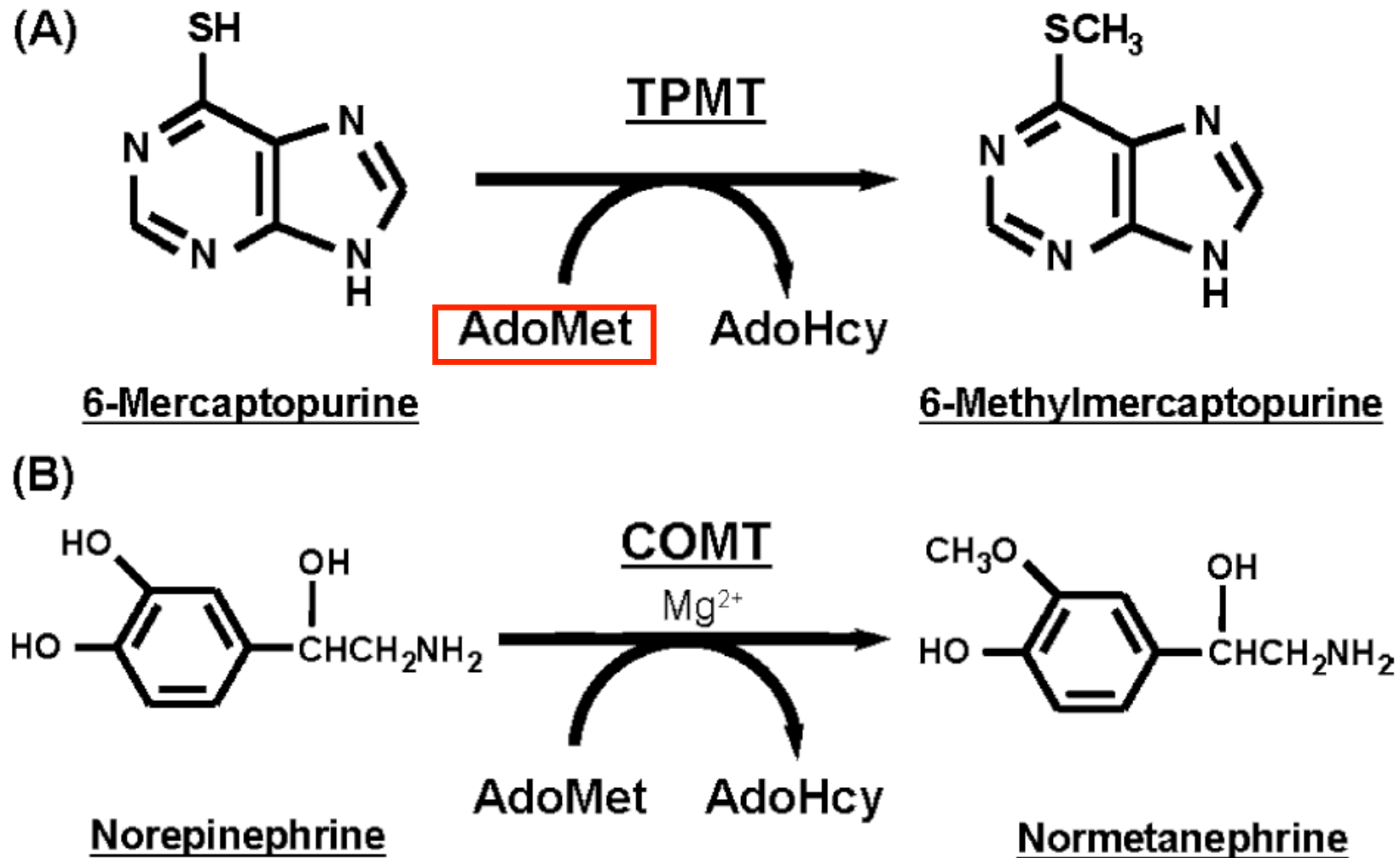
<sup>a</sup>Determined using Fisher exact test. <sup>b</sup>Determined using Bonferroni-corrected P value. <sup>c</sup>P < 0.05 after Bonferroni multiple testing correction. Ototox., individuals with ototoxicity.



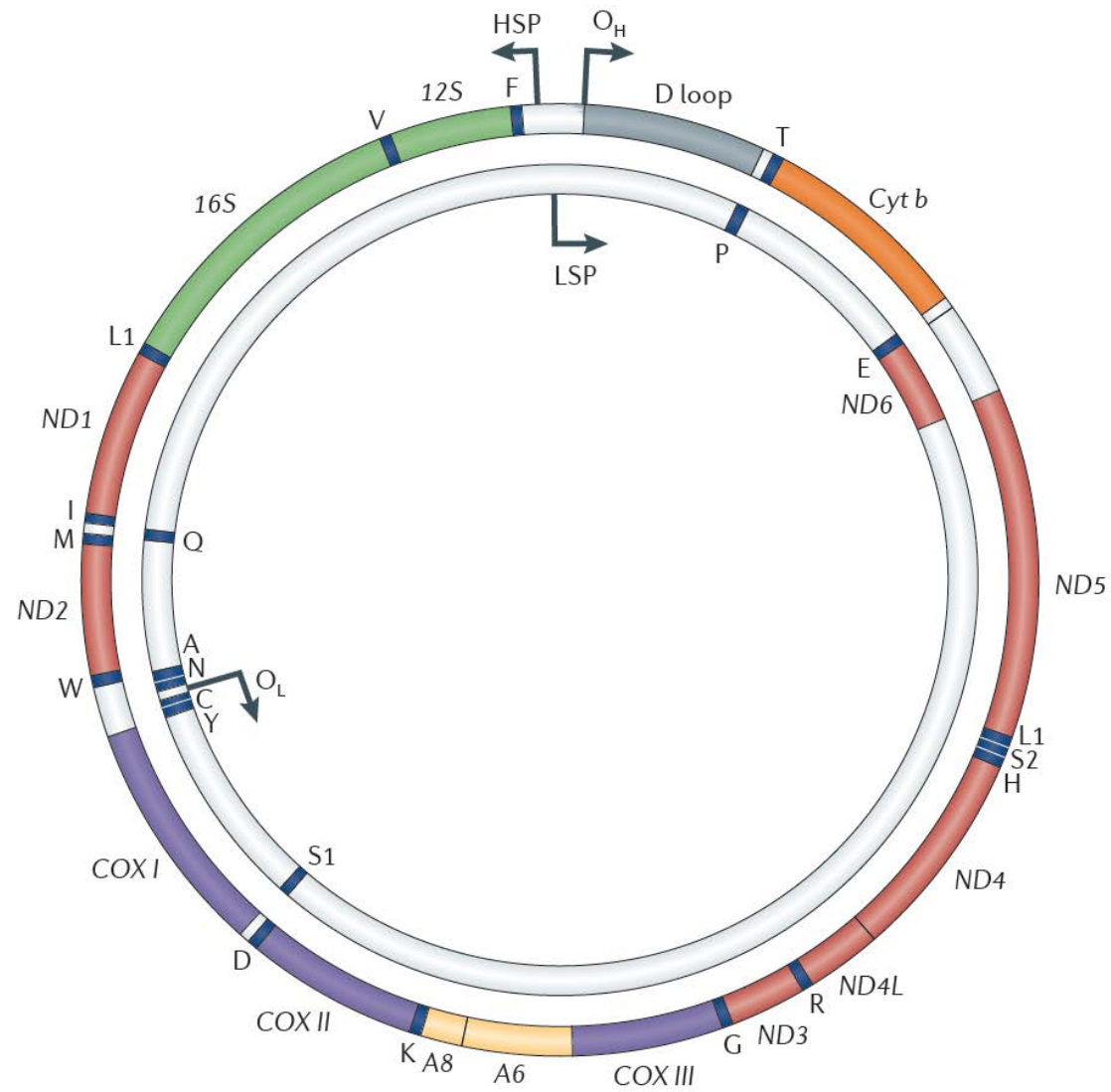
# Thiopurine Drug Metabolism



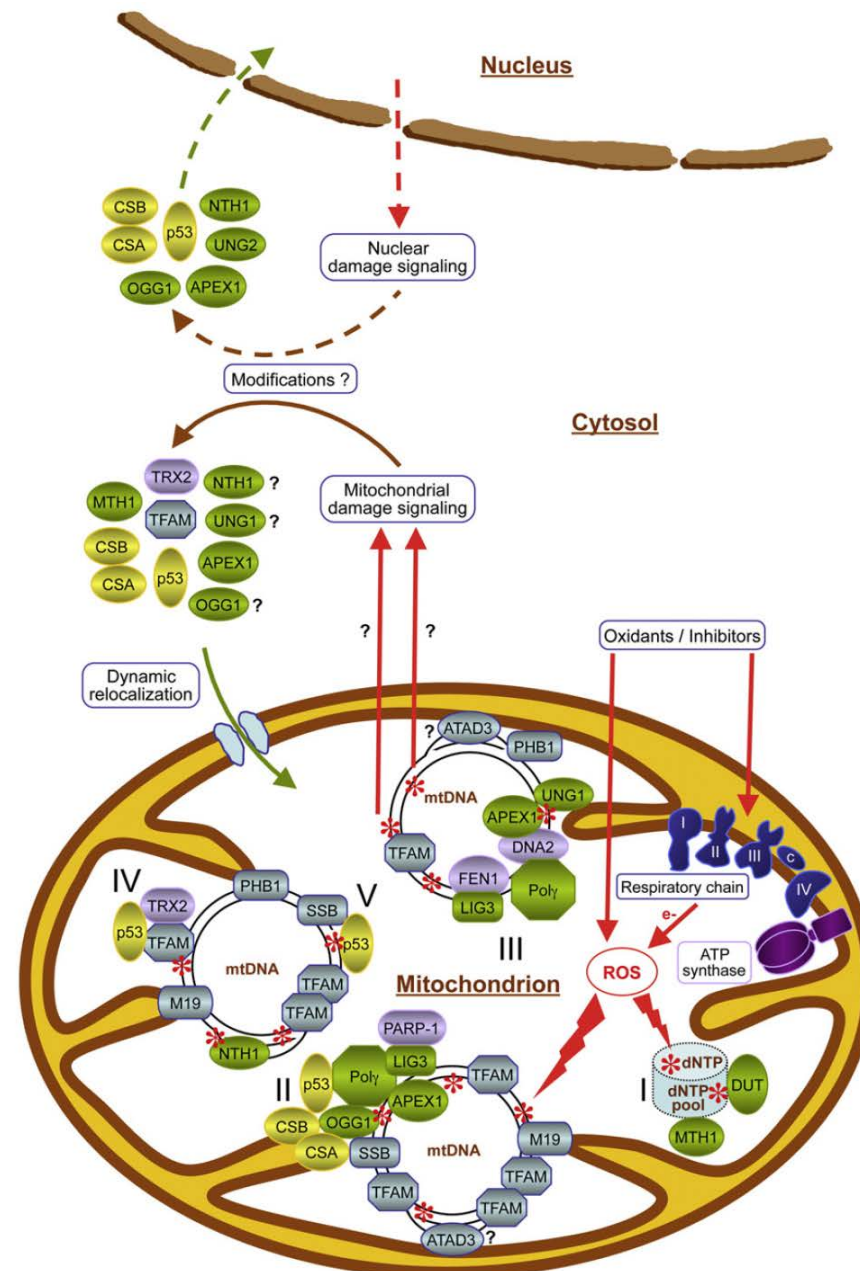
# TPMT and COMT-catalyzed Methylation



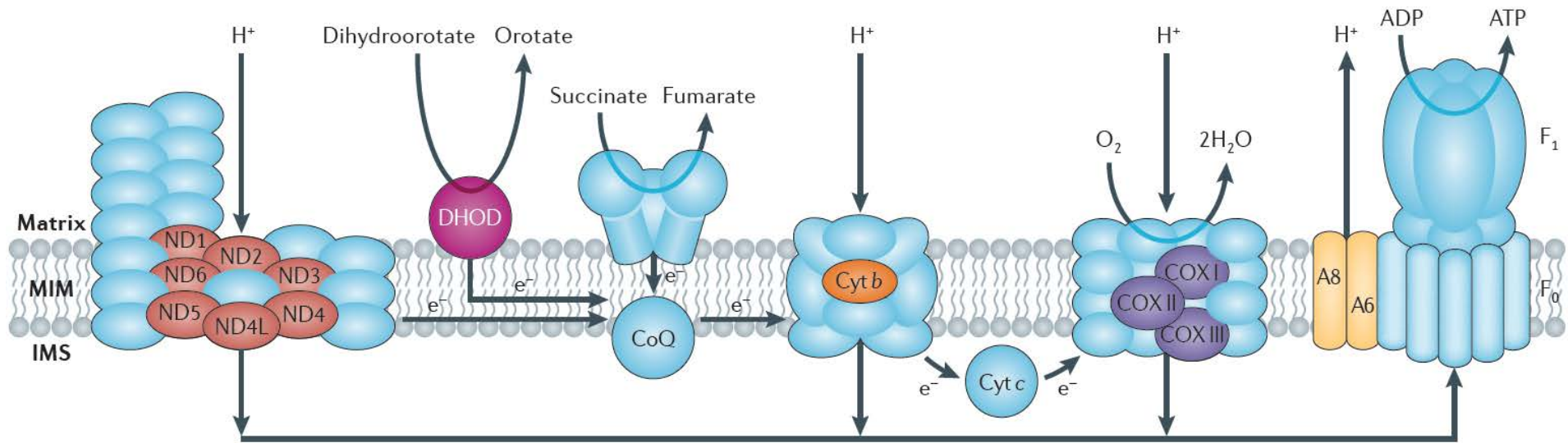
# The human mitochondrial genome



# Organization of oxidative DNA damage repair processes and factors in mammalian mitochondria

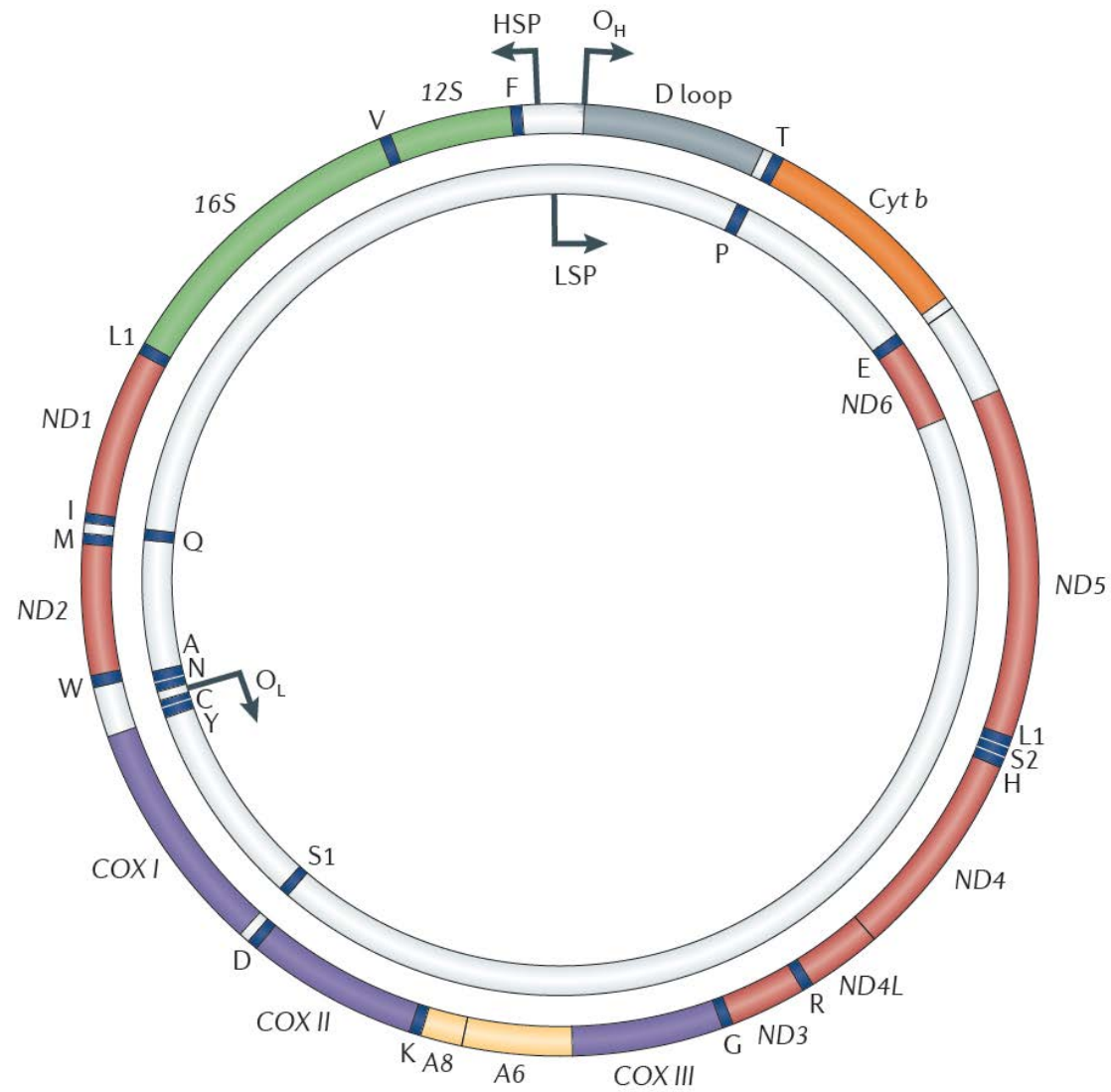


# Oxidative phosphorylation (OXPHOS) complexes



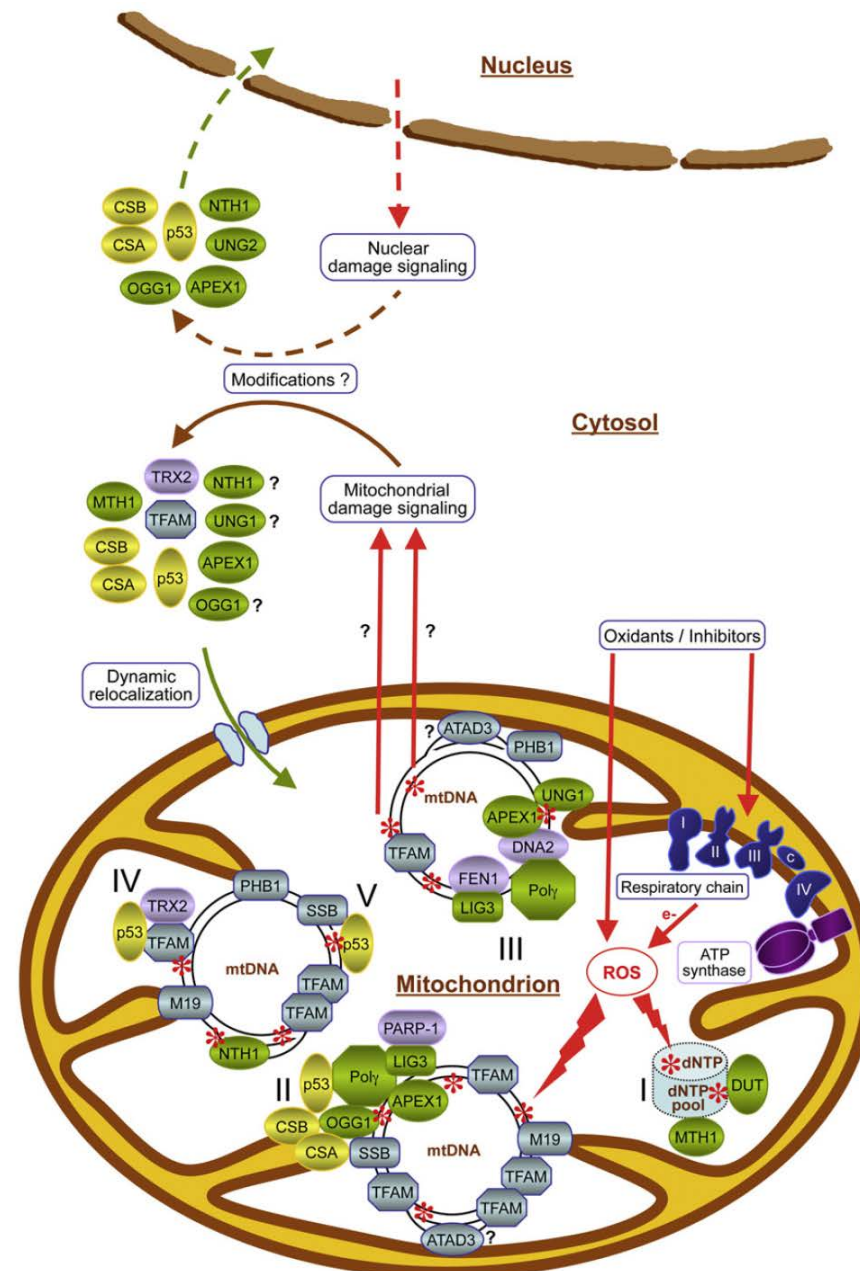
Polypeptides	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded subunits	7	0	1	3	2
nDNA-encoded subunits	~39	4	10	10	~14
Assembly proteins	~11	~2	~9	~30	~3

# The human mitochondrial genome





# Organization of oxidative DNA damage repair processes and factors in mammalian mitochondria





## Summary of mitochondrial nucleoid-associated proteins

Function	Mitochondrial nucleoid-associated proteins <sup>a</sup>
DNA replication, transcription, repair, recombination or packaging	<i>S. cerevisiae</i> : Abf2, Rim1, Rpo41, Mip1, Mgm101, Sls1, Pif1 [69] <i>H. sapiens</i> : TFAM, mtSSB, mtRNApol [24], POLG, POLG2 [24], PDIP38 [70], BRCA1, TFB2M [24], TWINKLE helicase, SUPV3L1 helicase [24], DHX30 helicase [24], HMGA1 [71], LRP130 [24,70] <i>X. leavis</i> : mtTFA, mtSSB
Heat shock proteins, proteases	<i>S. cerevisiae</i> : Hsp60, Ssc1, Ecm10 [42], Hsp10, Mdj1 [43], Mge1 [43] <i>H. sapiens</i> : Lon protease [70], HSP60 [24,70], CLPX protease [70], HSP90-β [70], HSP70 [24,70], PHB1 [24], PHB2 [24], TID1 [44] <i>X. leavis</i> : PHB2
Metabolic enzymes	<i>S. cerevisiae</i> : Aco1, Ald4, Arg5/6, Atp1, Cha1, Idh1, Idp1, Ilv5, Ilv6, Kgd1, Kgd2, Lpd1, Lsc1, Pda1, Pdb1 <i>H. sapiens</i> : AAT [70], ANT2 [24], ATPase β subunit [70], ATPase O subunit [70], CPS1 [24], CPT1A [24], DECR [70], HADHA [24,48], MDH [70], NDUFA9 [24], NDUFS1 [24], NDUFS3 [24], PDX3 [70], SHMT2 [24] <i>X. leavis</i> : ANT1, PDC-E2, BCKAD-E2 <i>V. radiata</i> : ATPase α subunit [25]
Cytoskeletal components	<i>H. sapiens</i> : actin [24], vimentin [24] <i>V. radiata</i> : actin [25]
Other	<i>S. cerevisiae</i> : Mnp1, Yhm2 <i>H. sapiens</i> : mitofilin [24], SCCA1 [70], SCCA2 [70], MGC5352 [24], ATAD3 [24,48]

## mtDNA mutations in human primary respiratory chain disorders

Gene product	Number of mutations	Main disorder
<b>rRNAs</b>		
12S rRNA	5	Deafness
16S rRNA	1	Atypical MELAS
<b>Polypeptides</b>		
ATP synthase 6	13	NARP or MILS
ATP synthase 8	2	Various
COX I	10	Various
COX II	8	Various
COX III	6	Myopathy
Cytochrome <i>b</i>	21	Sporadic myopathy
ND1	16	MELAS; LHON
ND2	3	Various
ND3	5	Leigh's syndrome
ND4	5	LHON
ND4L	1	LHON
ND5	12	MELAS
ND6	11	LHON

COX: cytochrome oxidase

LHON: Leber hereditary optic neuropathy

MELAS: mito encephalomyopathy lactic acidosis & stroke-like episodes

MERRF: myoclonus epilepsy & ragged red fibres

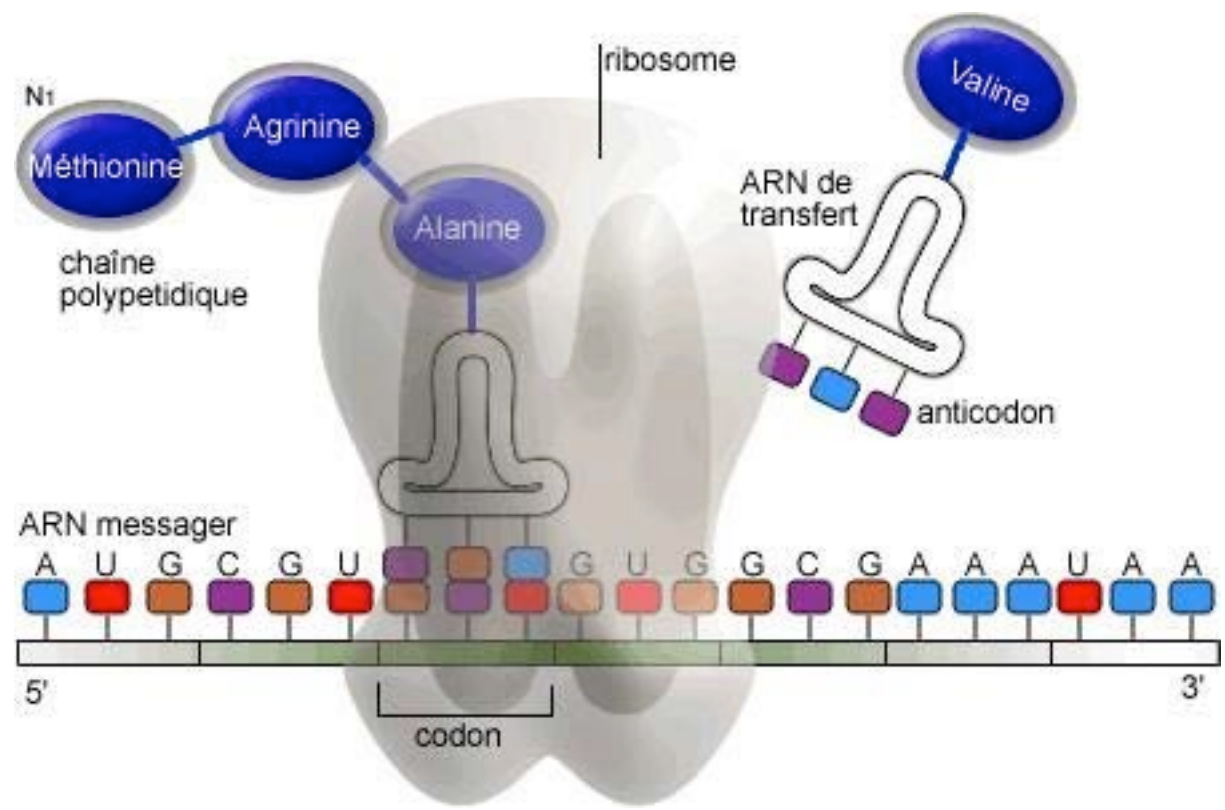
MILS: maternally inherited Leigh syndrome

NARP: neuropathy ataxia & retinitis pigmentosa

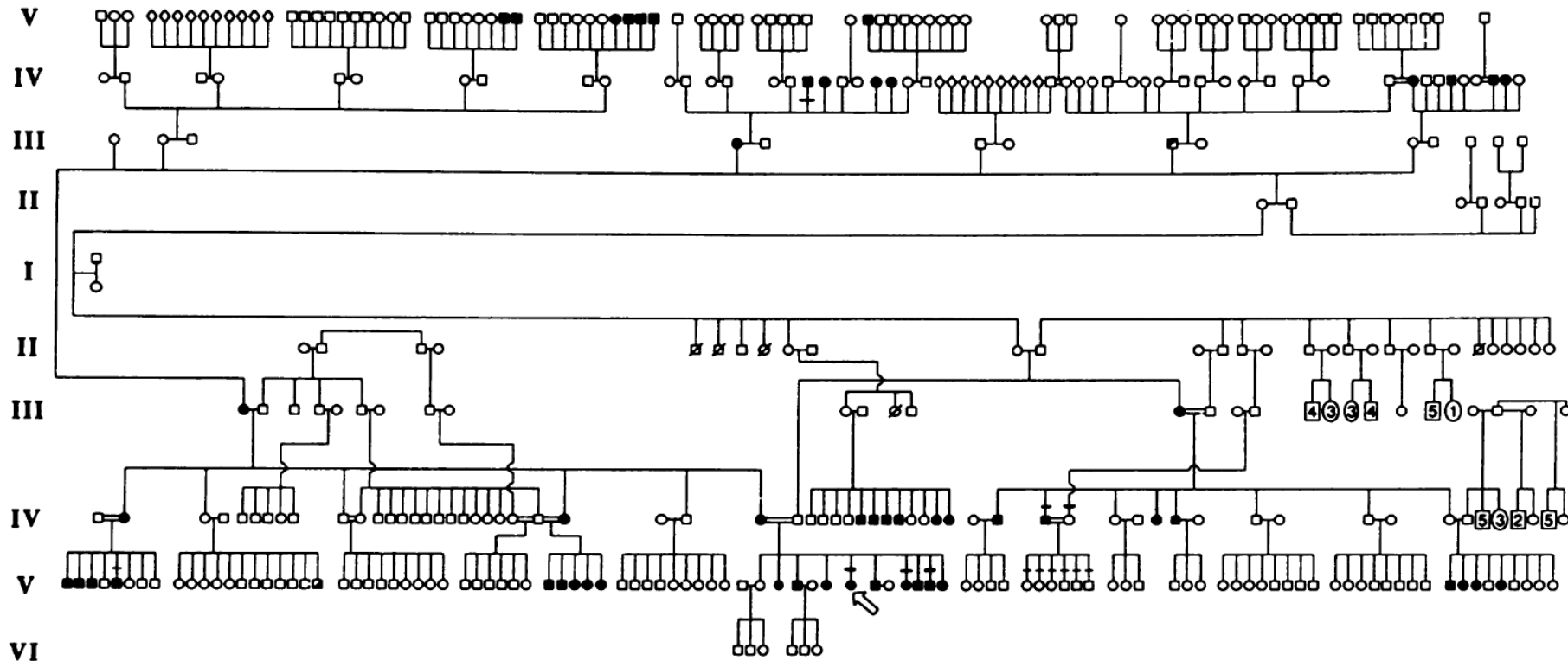
ND: NADH-ubiquinone oxidoreductase

PEO: progressive external ophthalmoplegia

Gene product	Number of mutations	Main disorder
<b>tRNAs</b>		
tRNA <sup>Ala</sup>	3	Myopathy
tRNA <sup>Arg</sup>	2	Various
tRNA <sup>Asn</sup>	5	Myopathy
tRNA <sup>Asp</sup>	2	Various
tRNA <sup>Cys</sup>	3	Various
tRNA <sup>Gln</sup>	3	Various
tRNA <sup>Glu</sup>	7	Reversible respiratory chain deficiency
tRNA <sup>Gly</sup>	3	Various
tRNA <sup>His</sup>	4	Various
tRNA <sup>Ile</sup>	14	PEO
tRNA <sup>Leu(CUN)</sup>	8	Myopathy
tRNA <sup>Leu(UUR)</sup>	23	MELAS
tRNA <sup>Lys</sup>	14	MERRF
tRNA <sup>Met</sup>	2	Various
tRNA <sup>Phe</sup>	14	Myopathy
tRNA <sup>Pro</sup>	5	Multisystem
tRNA <sup>Ser(AGY)</sup>	4	Myopathy
tRNA <sup>Ser(UCN)</sup>	12	Myopathy; deafness
tRNA <sup>Thr</sup>	2	Various
tRNA <sup>Trp</sup>	12	Encephalomyopathy
tRNA <sup>Tyr</sup>	4	Myopathy
tRNA <sup>Val</sup>	6	Multisystem

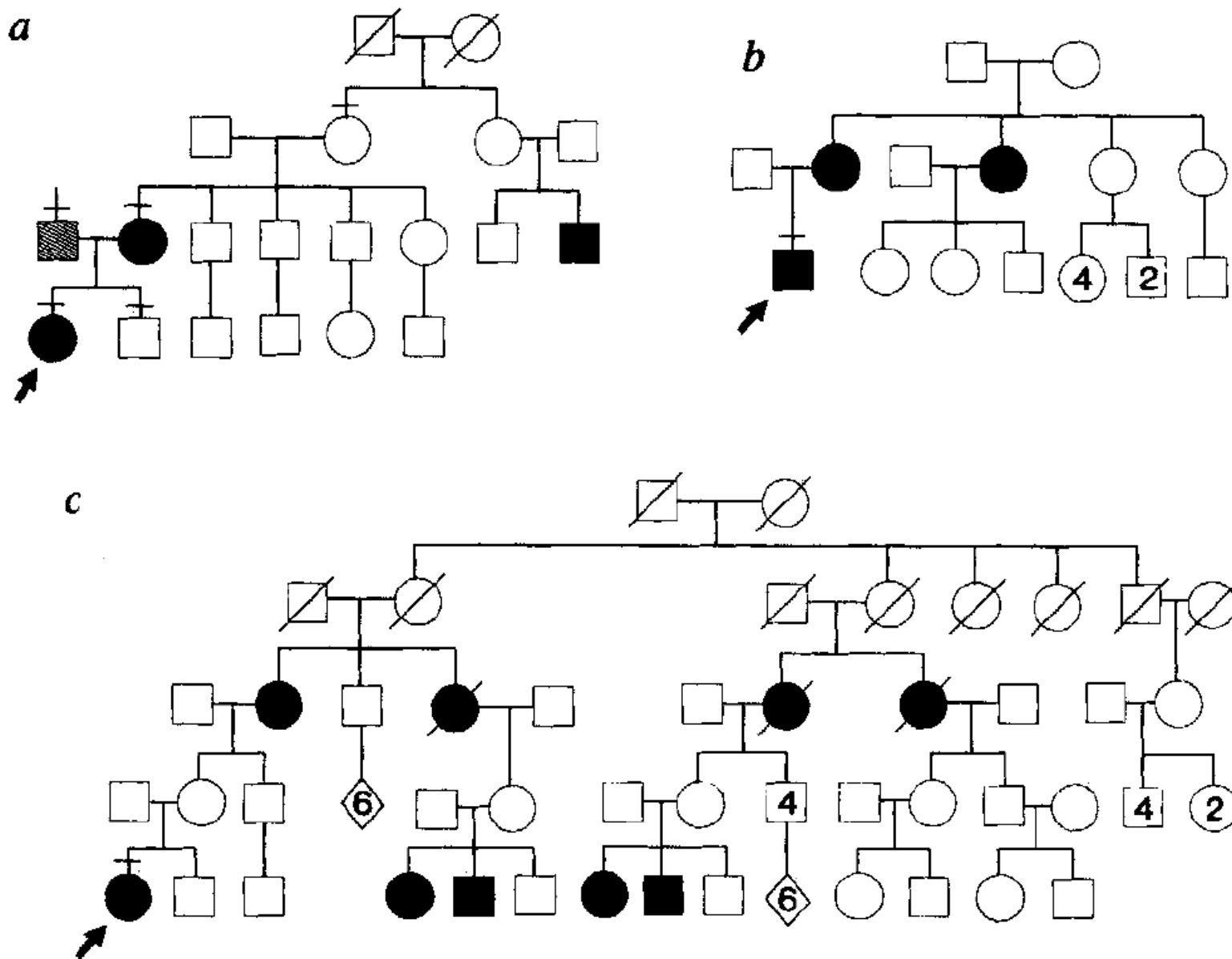


# Israeli-Arab pedigree with sensorineural deafness



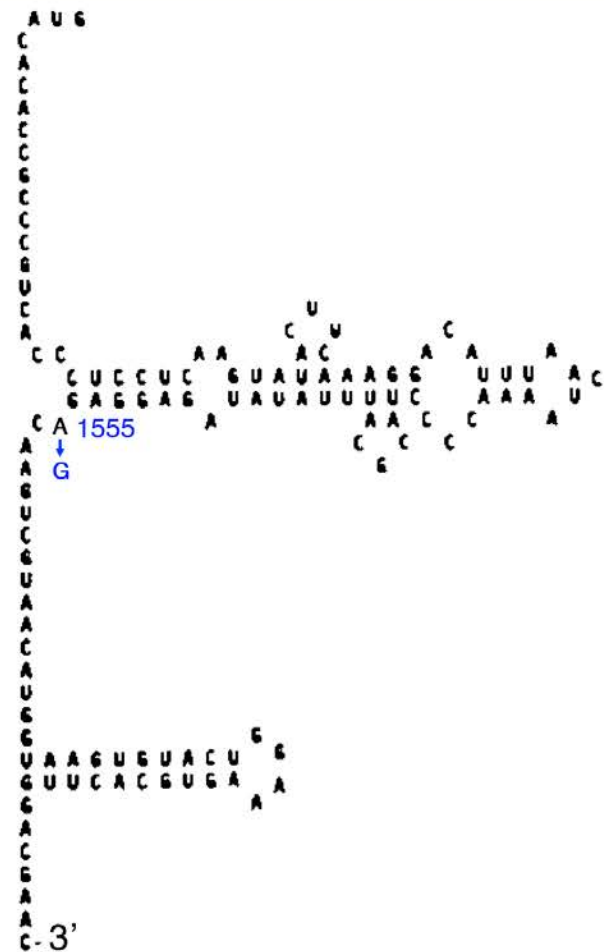
- |   |                                      |
|---|--------------------------------------|
| ◻◻ Unaffected male, female                  | ◻◻ Consanguineous mating or marriage |
| ● Deaf, onset in infancy or early childhood | ◊ Sex unknown                        |
| ■ Deaf, onset in adulthood                  | ⊕ Underwent audiometric tests        |
| ∕ Died, no information available            | ● Proband                            |
|   | ↖                                    |

# Three pedigrees with maternally-transmitted aminoglycoside-induced deafness

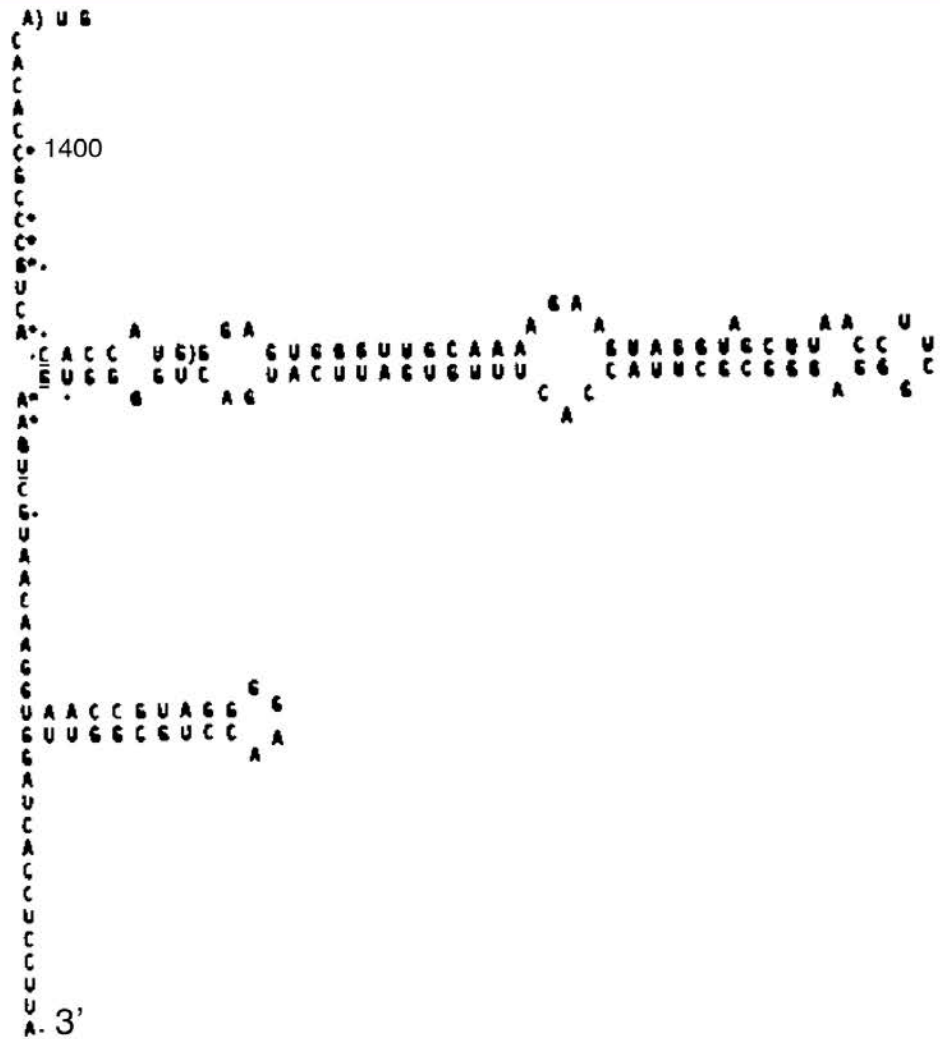


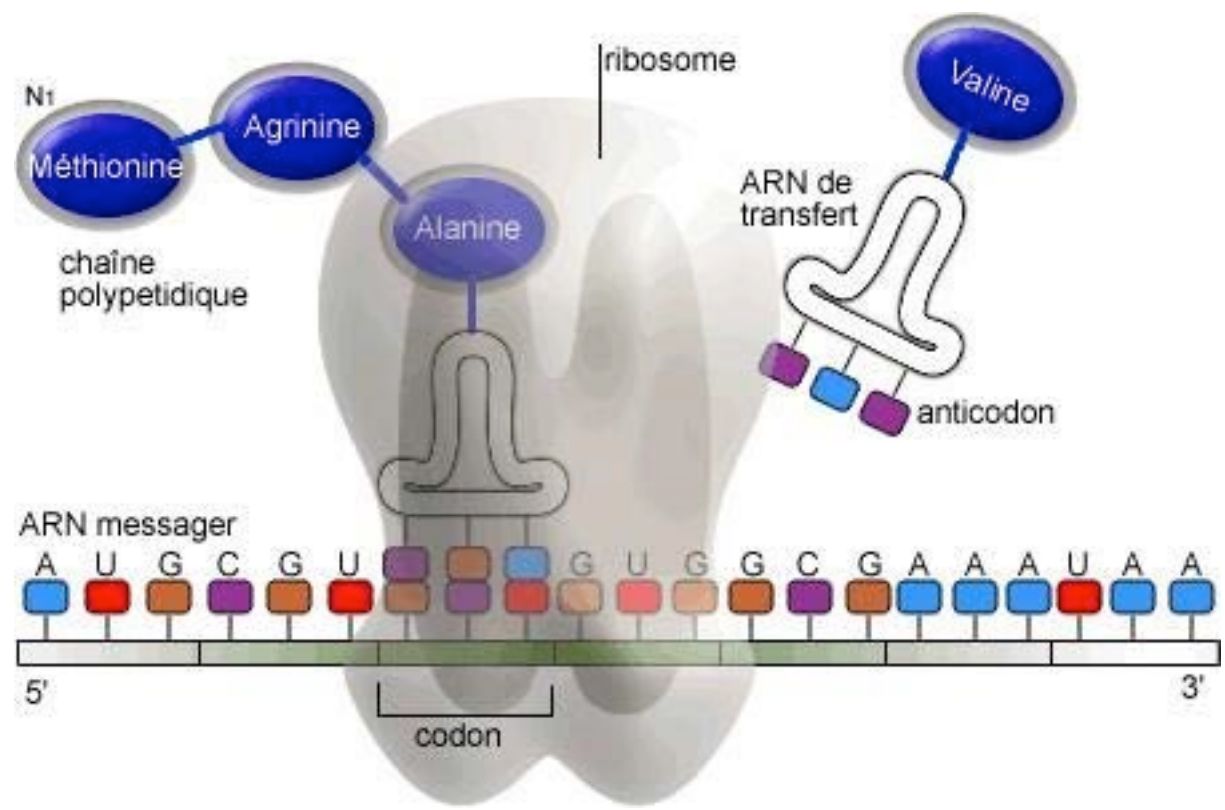
The 3'-end of human mitochondrial 12S rRNA and the corresponding region of *E. coli* 16S rRNA, showing the site of the 1555A to G mutation in the four pedigrees with maternally-transmitted deafness

3' end of human mitochondrial 12S rRNA



3' end of *E. coli* 16S rRNA







**Mitochondrial ribosomal RNA  
mutation associated with both  
antibiotic-induced and non-  
syndromic deafness**

*nature genetics* volume 4 july 1993

Toni R. Prezant<sup>1</sup>, John V. Agapian<sup>1</sup>, M. Charlotte Bohlman<sup>1</sup>, Xiangdong Bu<sup>1</sup>, Sitki Öztas<sup>1</sup>,  
Wei-Qin Qiu<sup>2</sup>, Kathleen S. Arnos<sup>3</sup>, Gino A. Cortopassi<sup>4</sup>, Lutfi Jaber<sup>5</sup>, Jerome I. Rotter<sup>1</sup>,  
Mordechai Shohat<sup>5</sup> & Nathan Fischel-Ghodsian<sup>1</sup>

4174–4179 *Nucleic Acids Research*, 1993, Vol. 21, No. 18

**A molecular basis for human hypersensitivity to  
aminoglycoside antibiotics**

Tim Hutchin, Ian Haworth<sup>1</sup>, Koichiro Higashi<sup>2</sup>, Nathan Fischel-Ghodsian<sup>3</sup>, Mark Stoneking<sup>4</sup>,  
N.Saha<sup>5</sup>, Cathy Arnos<sup>6</sup> and Gino Cortopassi\*

*American Journal of Otolaryngology*, Vol 14, No 6 (November-December), 1993: pp 399-403

**Mitochondrial Ribosomal RNA Gene Mutation in a Patient  
With Sporadic Aminoglycoside Ototoxicity**

NATHAN FISCHEL-GHODSIAN, MD, TONI R. PREZANT, PhD,  
XIANGDONG BU, MD, AND SITKI ÖZTAS, MD

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **223**, 496–501 (1996)

**Mutant mtDNA at 1555 A to G in 12S rRNA Gene and  
Hypersusceptibility of Mitochondrial Translation to Streptomycin Can Be  
Co-Transferred to  $\rho^0$  HeLa Cells**

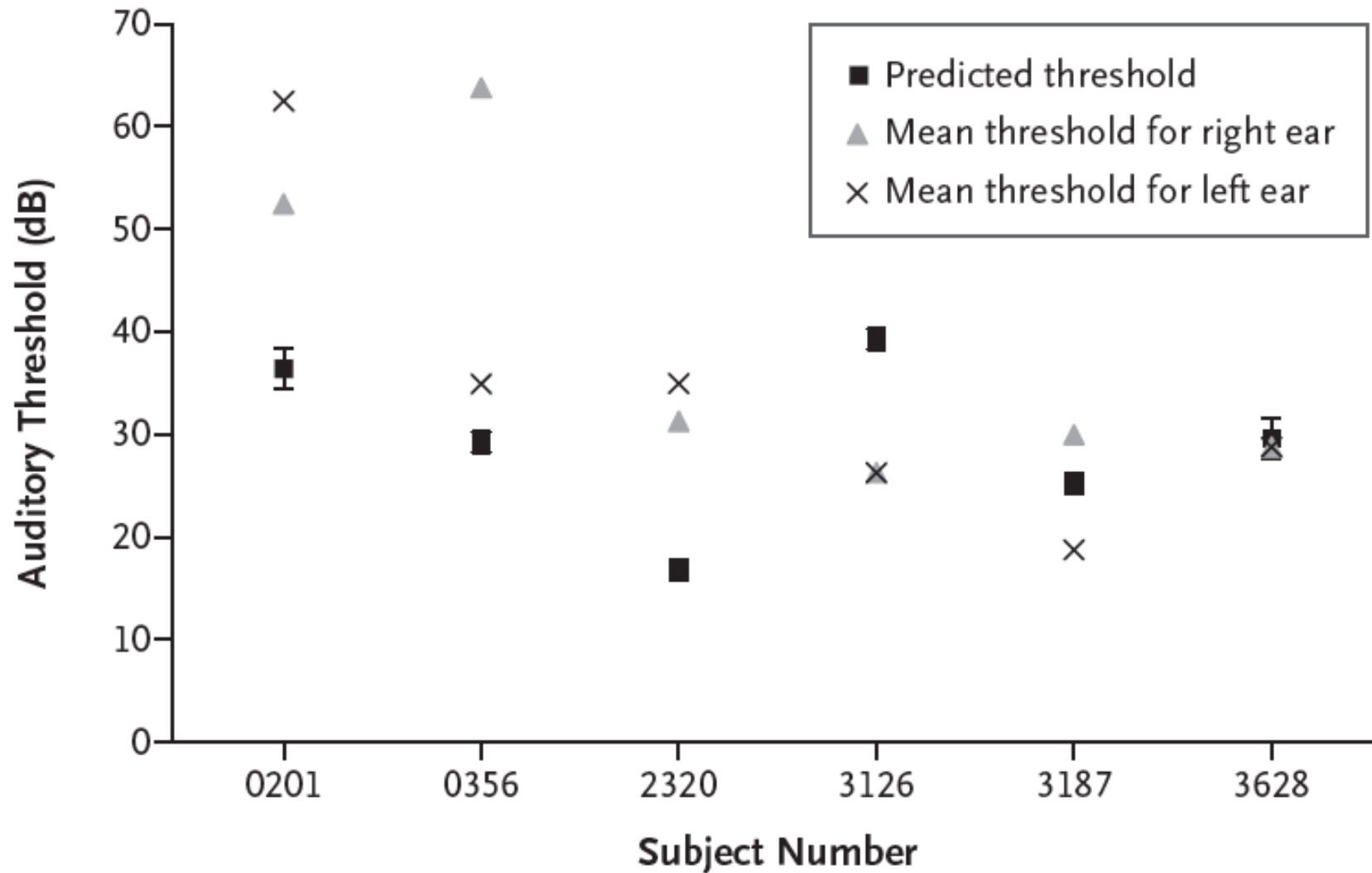
Kimiko Inoue,\* Daisaku Takai,\* Aki Soejima,\* Kotoyo Isobe,\* Tatsuya Yamasoba,†  
Yoshitomo Oka,‡ Yu-ichi Goto,§ and Jun-Ichi Hayashi\*.<sup>1</sup>

*American Journal of Otolaryngology*, Vol 18, No 3 (May-June), 1997: pp 173-178

**Mitochondrial Gene Mutation Is a Significant  
Predisposing Factor in Aminoglycoside Ototoxicity**

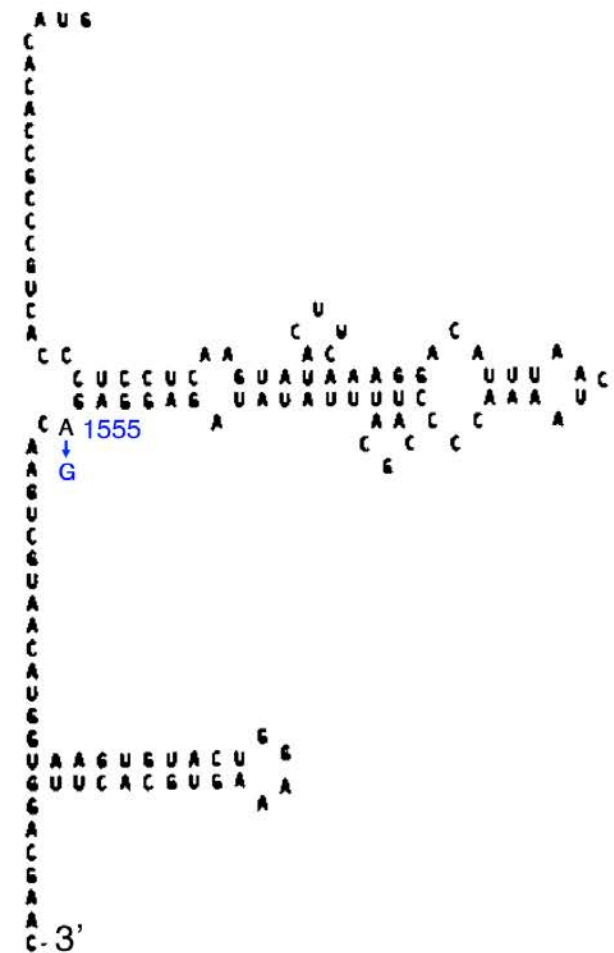
*Nathan Fischel-Ghodsian, MD, Toni R. Prezant, PhD, William E. Chaltraw, BS,  
Kimberly A. Wendt, MS, Ralph A. Nelson, MD, Kathleen S. Arnos, PhD,  
and Rena E. Falk, MD*

# Mean auditory thresholds for six subjects with the m.1555A→G mutation, as compared with predicted thresholds

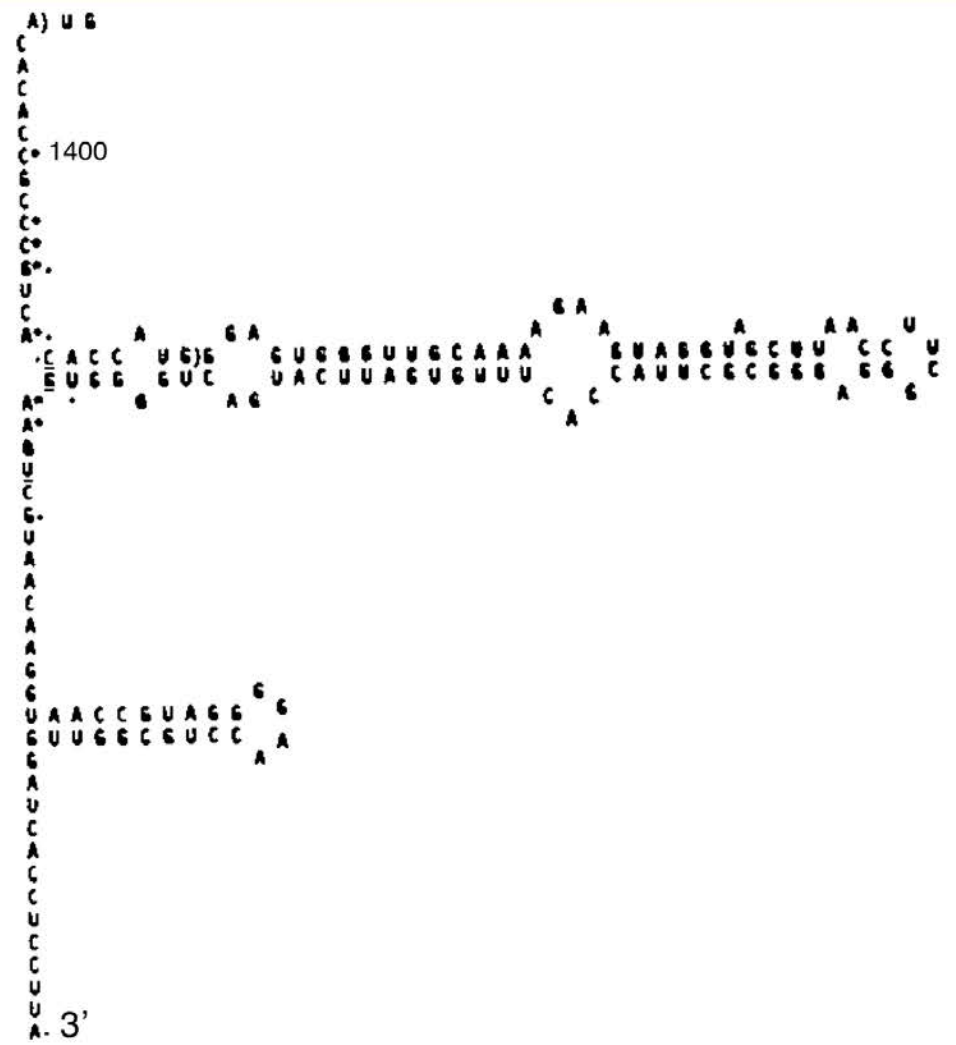


The 3'-end of human mitochondrial 12S rRNA and the corresponding region of *E. coli* 16S rRNA, showing the site of the 1555A to G mutation in the four pedigrees with maternally-transmitted deafness

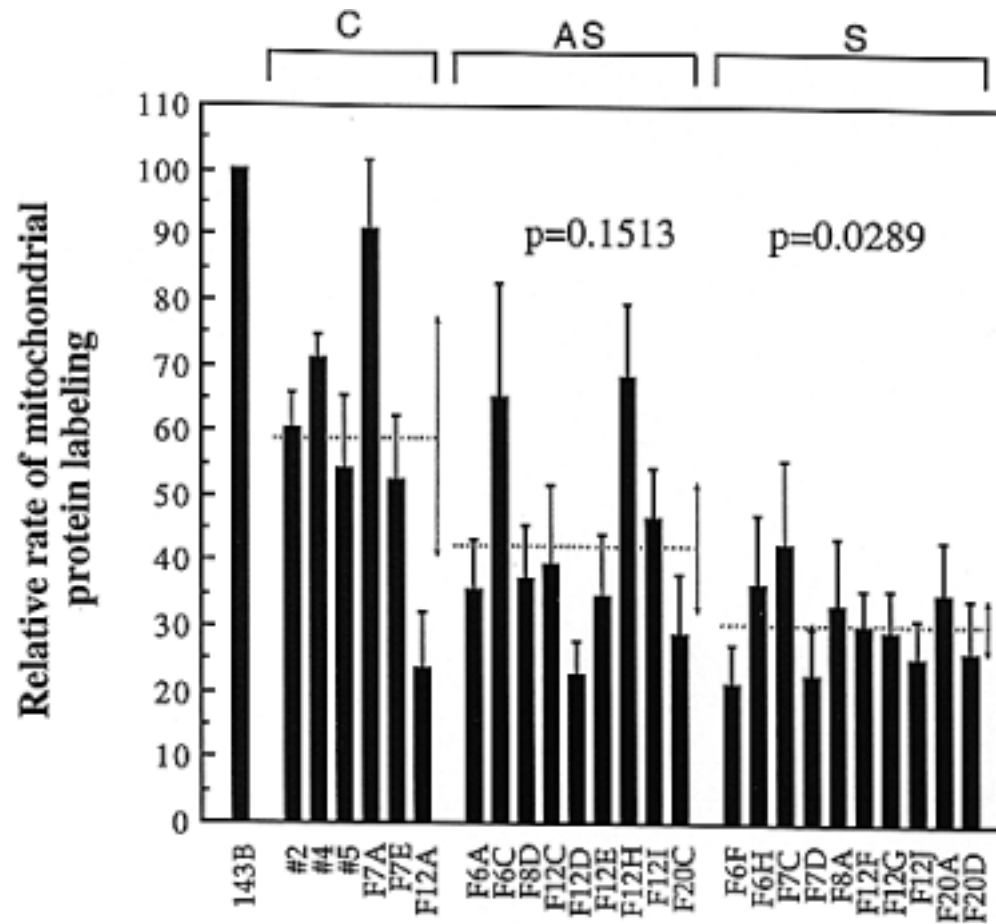
3' end of human mitochondrial 12S rRNA



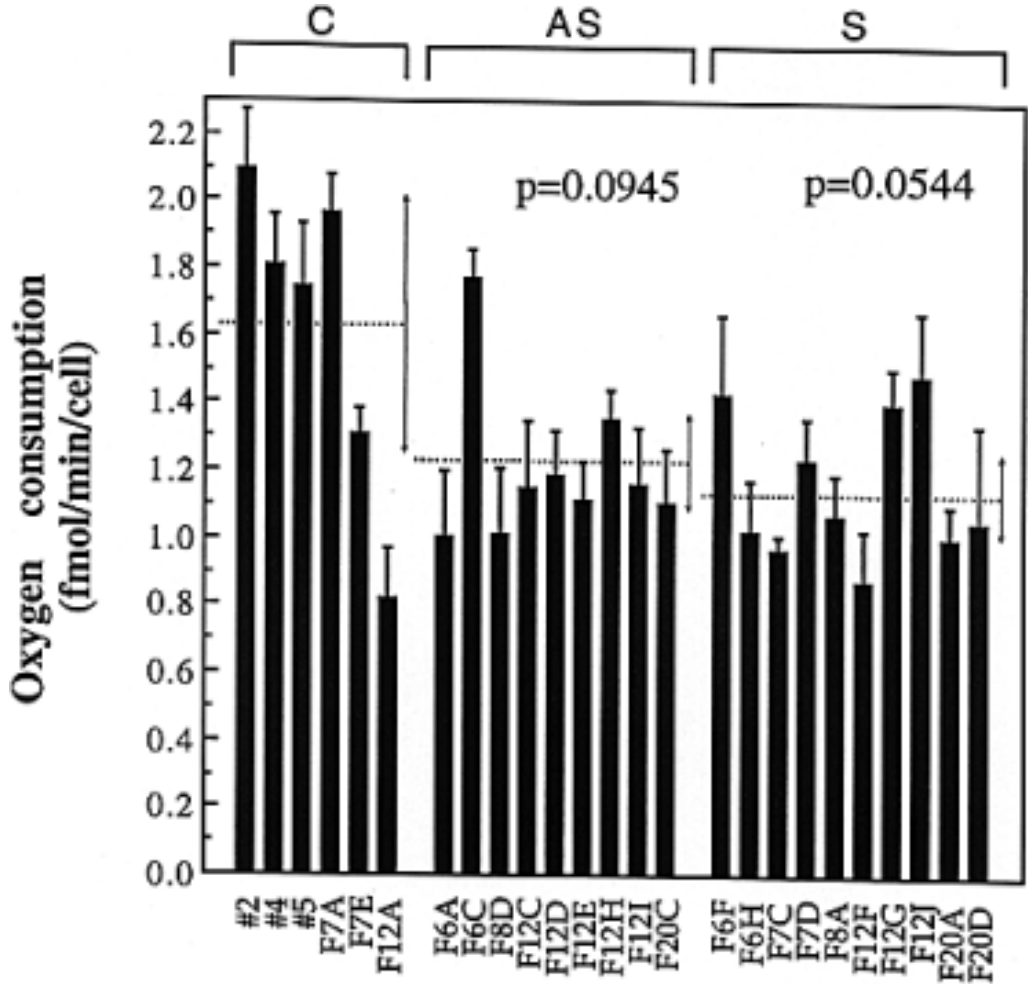
3' end of *E. coli* 16S rRNA



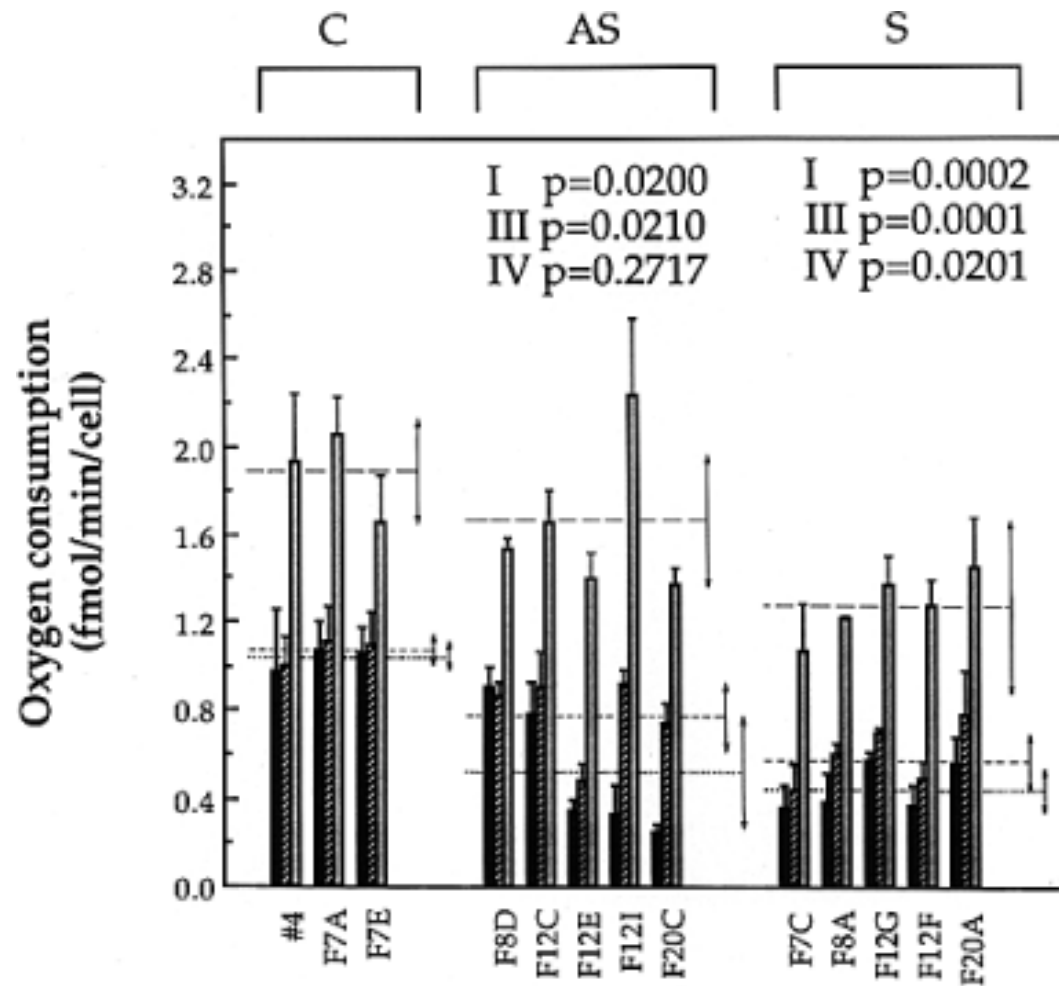
# Quantification of the rates of labeling of the mitochondrial translation products, after a 30 min [35S]methionine pulse, in different lymphoblastoid cell lines



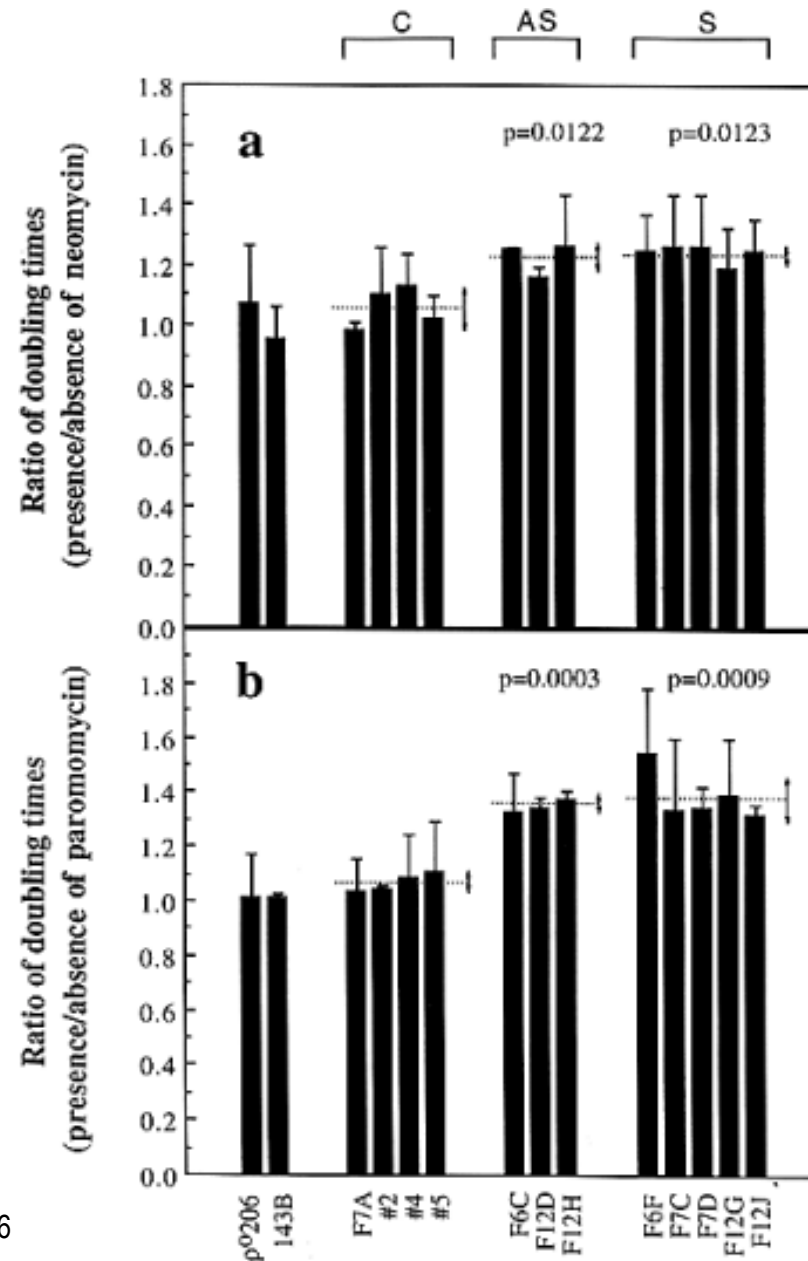
Average rates of oxygen consumption per cell measured in different lymphoblastoid cell lines



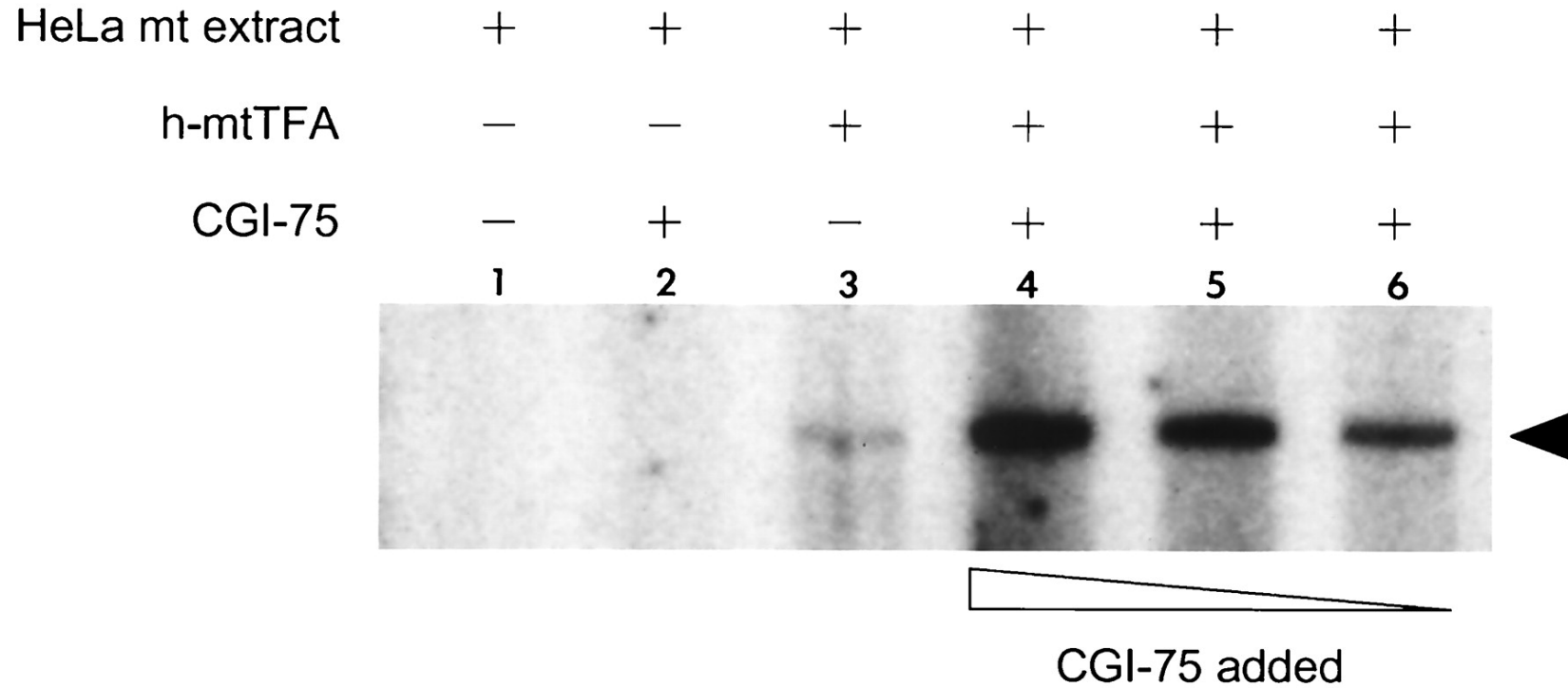
Activity of the enzymes of the mitochondrial respiratory chain in lymphoblastoid cell lines from symptomatic (S) and asymptomatic (AS) members of the Arab-Israeli family carrying the 12S rRNA 1555 mutation



Analysis of growth capacity of different lymphoblastoid cell lines in the presence of neomycin (a) or paromomycin (b)



# Recombinant human CGI-75 protein activates transcription from the human mitochondrial LSP

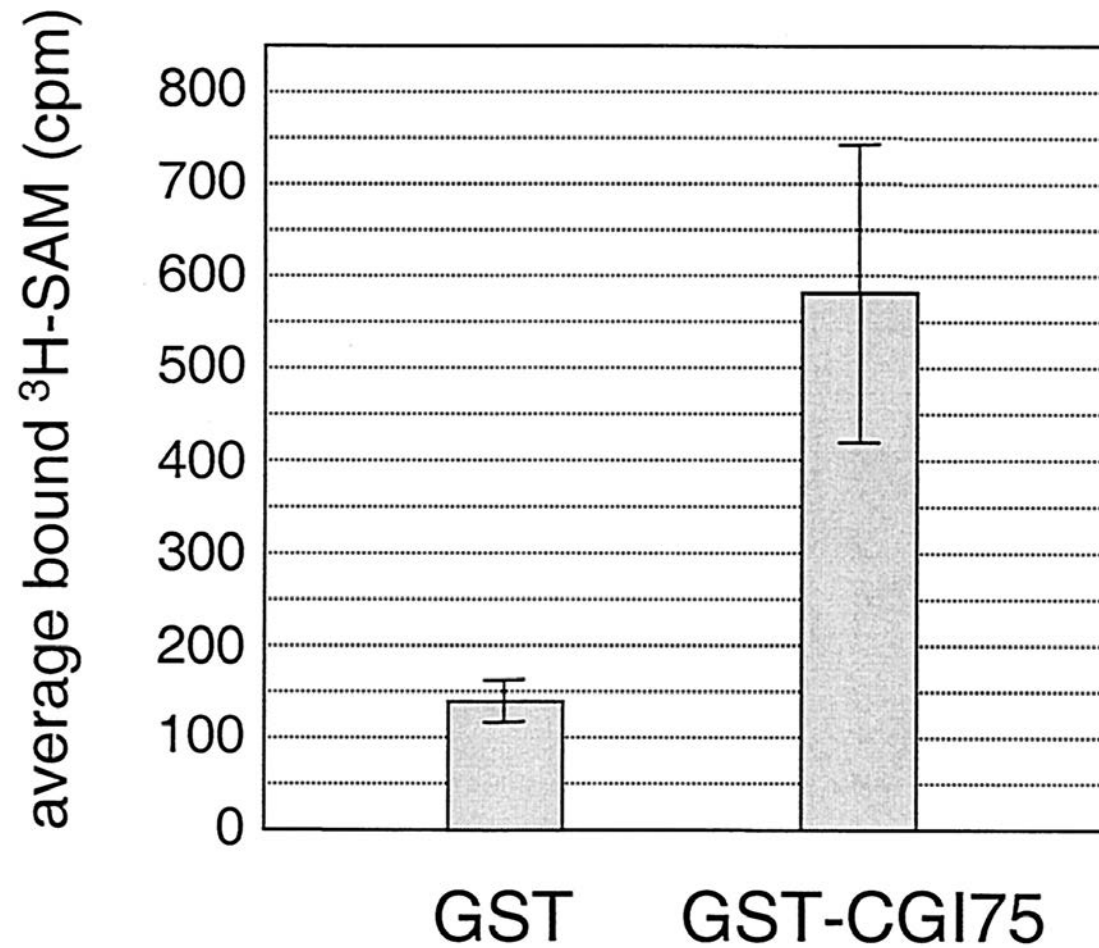




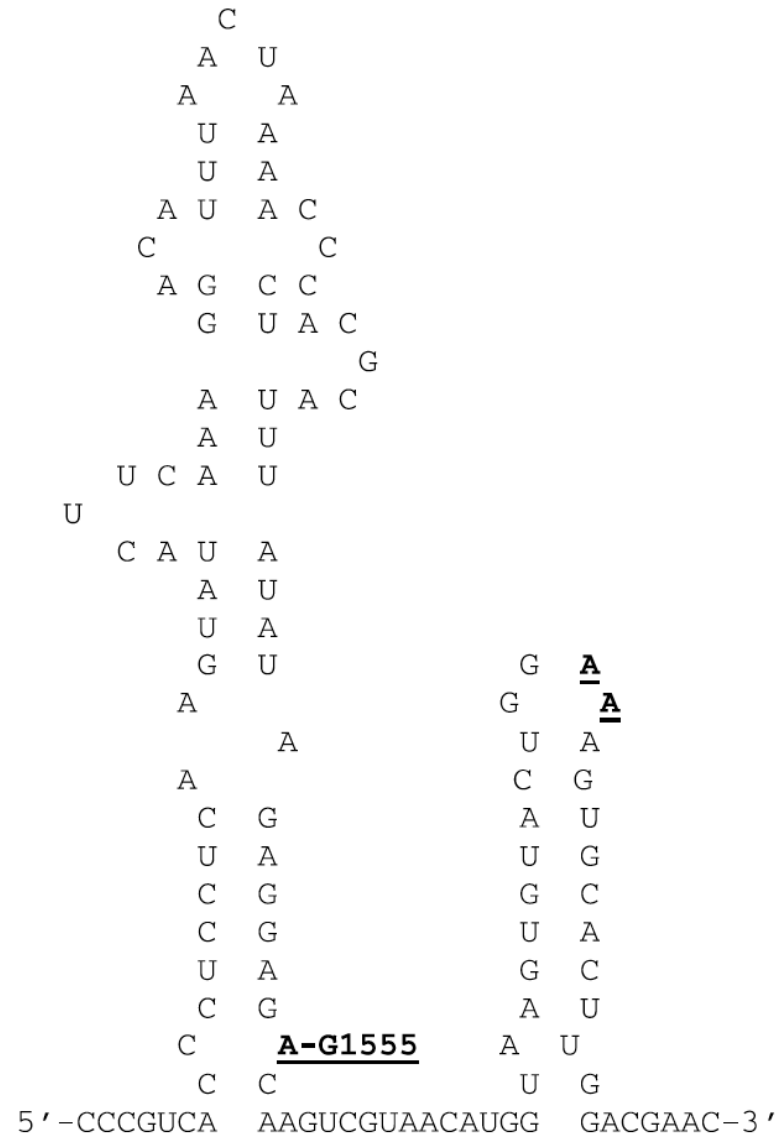
Representative proteins that exhibit a high degree of amino acid similarity to h-mtTFB and are members of an RNA (adenine-N6,N6)-dimethyltransferase superfamily

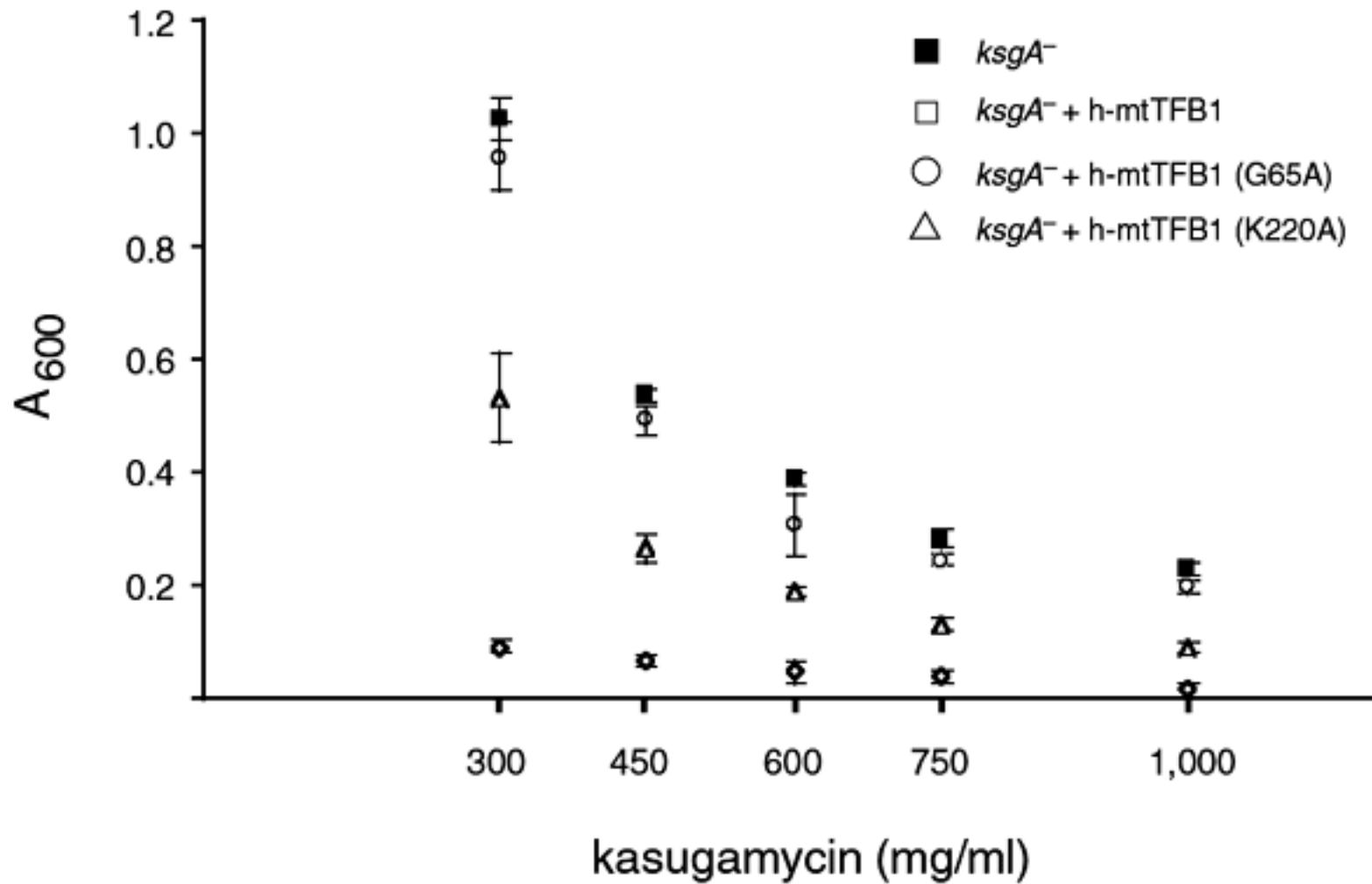
Gene product designation	Organism	E value	Accession no.
Dimethyladenosine transferase	<i>M. loti</i>	1e <sup>-44</sup>	NP_108091
	<i>Caulobacter crescentus</i>	7e <sup>-36</sup>	AAK23633
rRNA (adenosine-N6,N6)-dimethylase	<i>Pseudomonas aeruginosa</i>	2e <sup>-30</sup>	H83571
	<i>S. pombe</i>	8e <sup>-10</sup>	T43249
KsgA/dimethyladenosine transferase	<i>Rickettsia prowazekii</i>	2e <sup>-29</sup>	005952
	<i>Bacillus subtilis</i>	2e <sup>-24</sup>	P37468
	<i>E. coli</i>	1e <sup>-22</sup>	P06992
Dimethyl adenosinetransferase-like protein	<i>Arabidopsis thaliana</i>	2e <sup>-15</sup>	BAB10912
Putative dimethyladenosine transferase	<i>Homo sapiens</i>	7e <sup>-12</sup>	NP_055288
Dimethylase	<i>S. pombe</i>	2e <sup>-10</sup>	T40240
Dim1p/dimethyladenosine transferase	<i>K. lactis</i>	2e <sup>-09</sup>	P78697
	<i>S. cerevisiae</i>	2e <sup>-09</sup>	NP_015057

## Recombinant h-mtTFB (CGI-75) binds S-adenosylmethionine

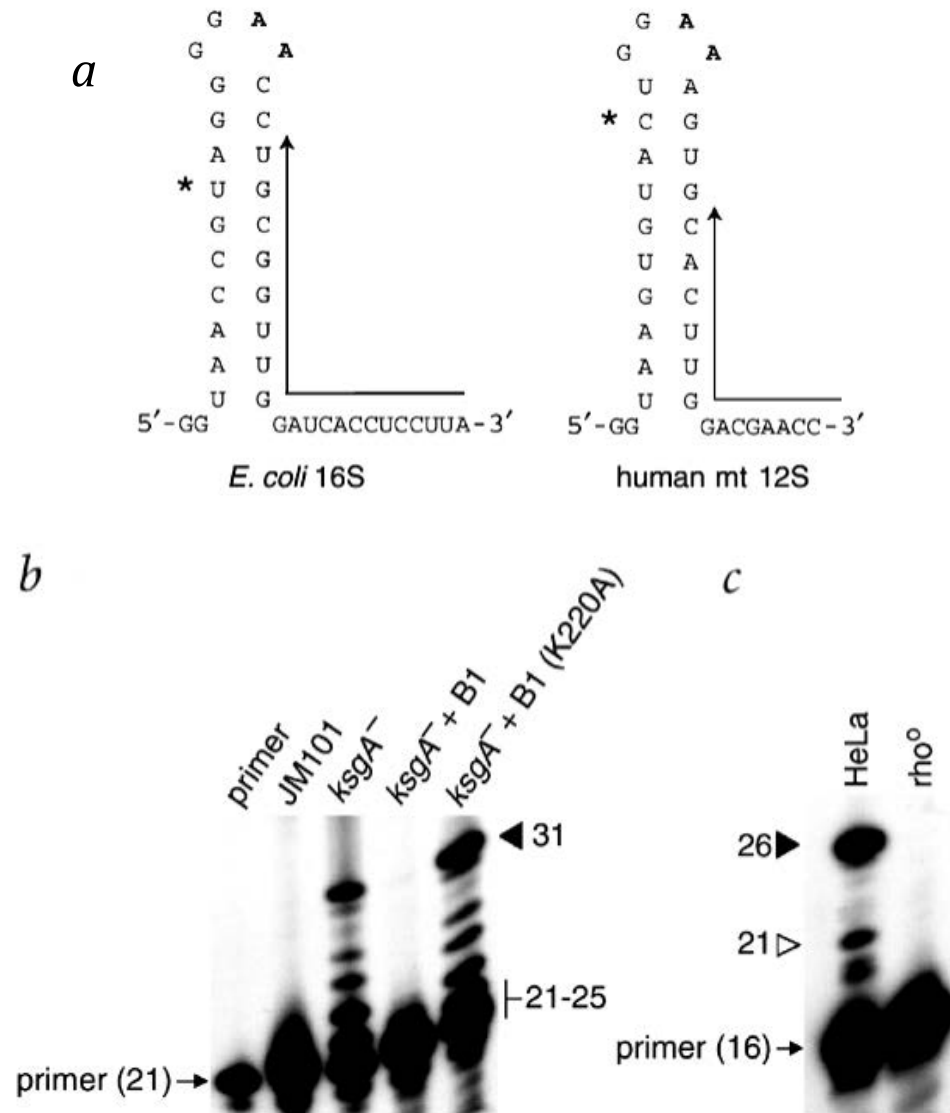


# Conserved stem-loop structures near the 3'-end of the human mitochondrial 12S rRNA

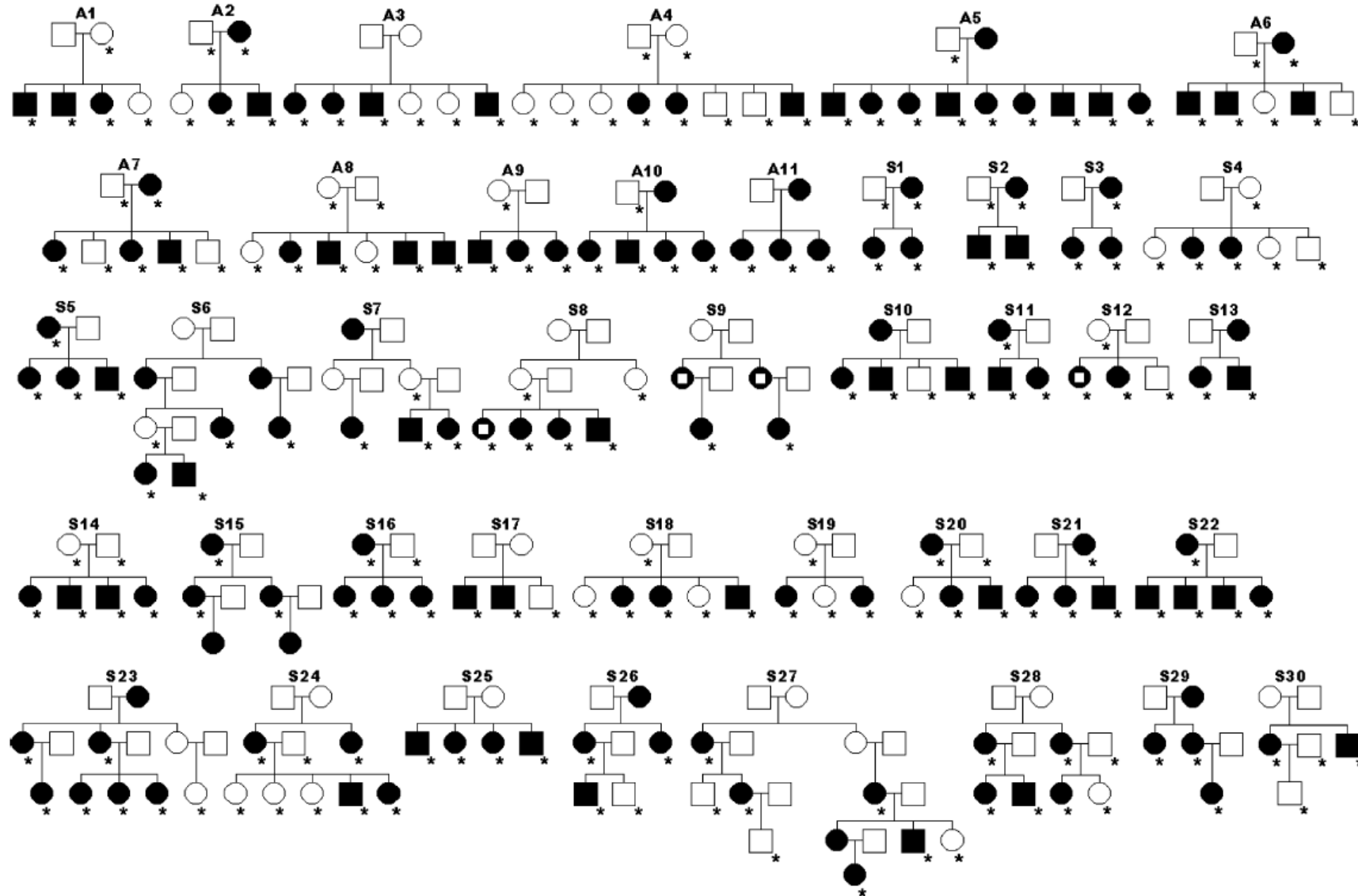


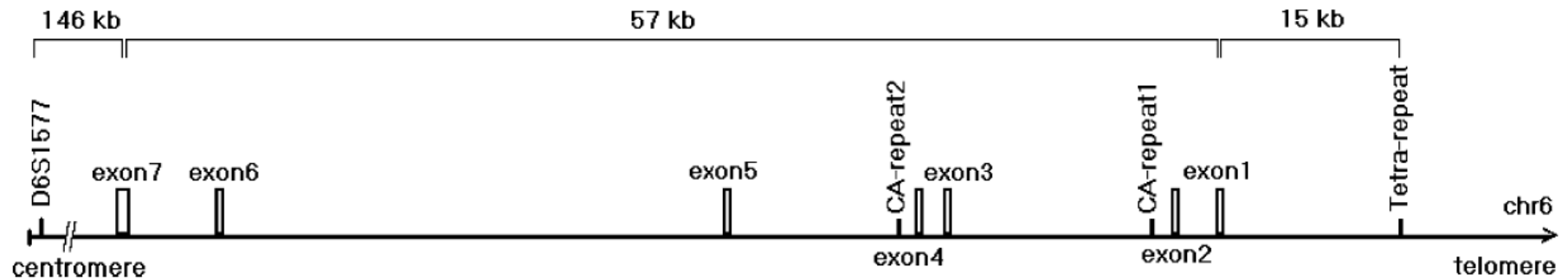


# h-mtTFB1 methylates bacterial rRNA at a conserved stem-loop that is similarly modified in human mitochondria



Families with a mitochondrial A1555G mutation and non-syndromic deafness of Arab-Israeli (families A1–11), and Spanish and Italian (families S1–30) origin





Location of polymorphic markers in the TFB1M gene.

Linkage and linkage disequilibrium analysis of polymorphic markers in the *TFB1M* gene

Polymorphic marker	Location	Multipoint LOD score	Two-point NPL score	TDT <sup>c</sup> (T:U)	TDT <sup>c</sup> $\chi^2$	TDT <sup>c</sup> <i>P</i>
Tetra-repeat <sup>a</sup>	15 kb 5' to TFB1M	1.4	1.2	8:17	3.24	NS <sup>b</sup>
CA-repeat 1 <sup>a</sup>	Intron 2	1.35	2.3	35:26	1.33	NS
CA-repeat 2 <sup>a</sup>	Intron 4	1.1	0.6	11:15	0.62	NS
D6S1577	146 kb 3' to TFB1M	1.1	1.0	8:25	8.76	0.003

<sup>a</sup> Predicted microsatellite repeats.

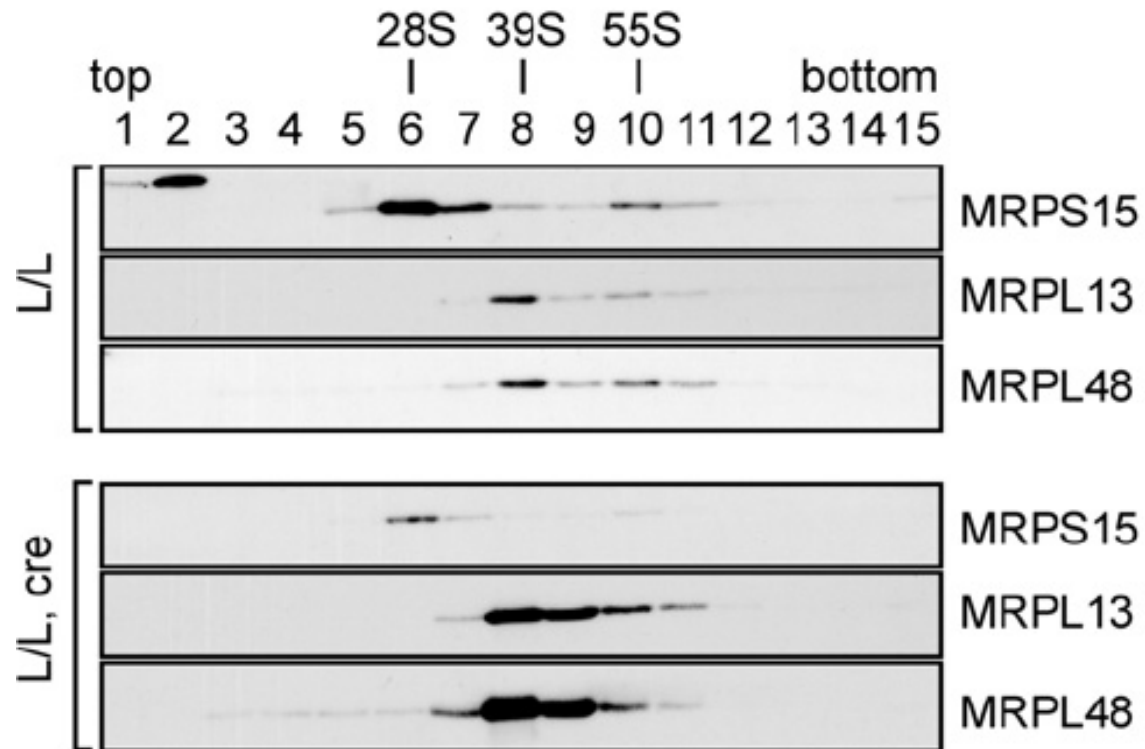
<sup>b</sup> No statistical difference (NS).

<sup>c</sup> Results for the most significant allele.

## Methylation of 12S rRNA Is Necessary for In Vivo Stability of the Small Subunit of the Mammalian Mitochondrial Ribosome

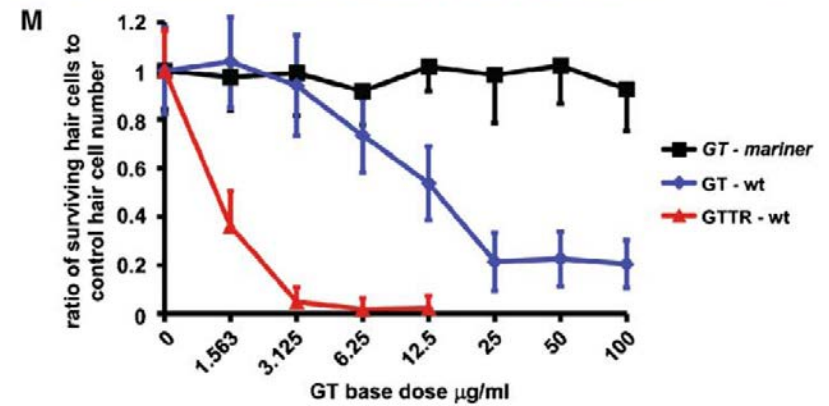
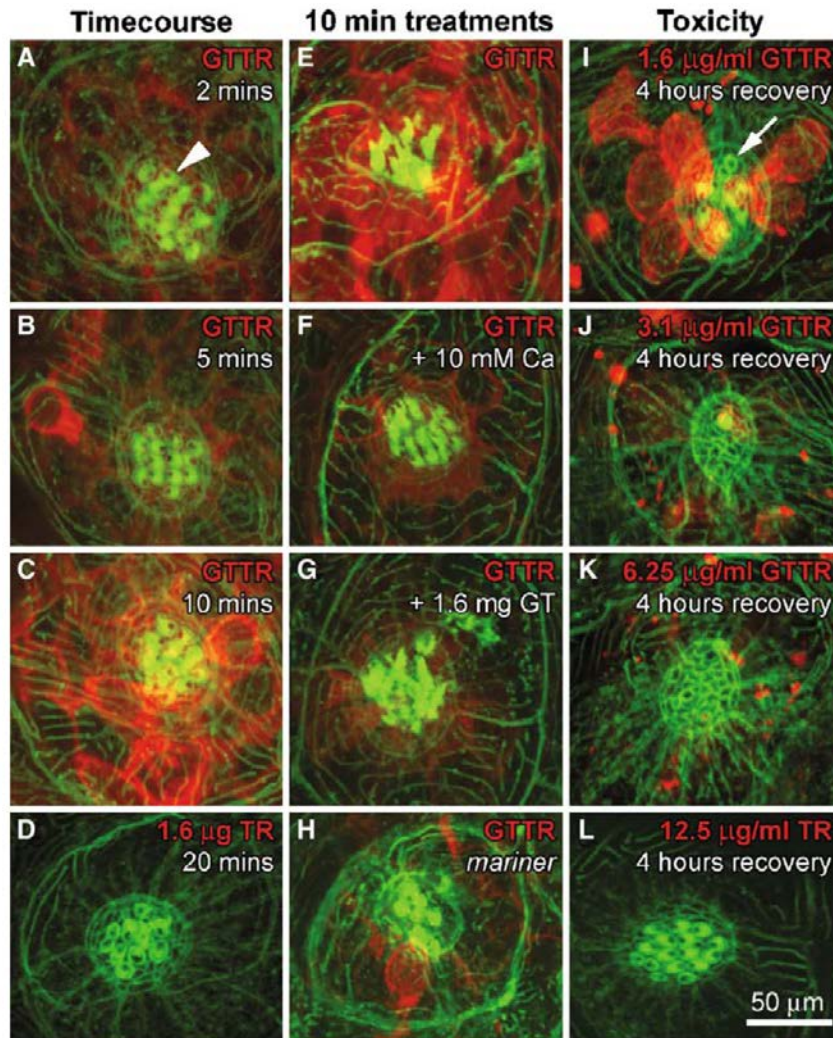
Metodi D. Metodiev,<sup>1,3</sup> Nicole Lesko,<sup>1</sup> Chan Bae Park,<sup>2</sup> Yolanda Cámara,<sup>1</sup> Yonghong Shi,<sup>1</sup> Rolf Wibom,<sup>1</sup> Kjell Hultenby,<sup>1</sup> Claes M. Gustafsson,<sup>1</sup> and Nils-Göran Larsson<sup>1,3,\*</sup>

Loss of 12S rRNA dimethylation results in reduced steady-state levels of the small ribosomal subunit in tissue-specific knockout of *Tfb1m*

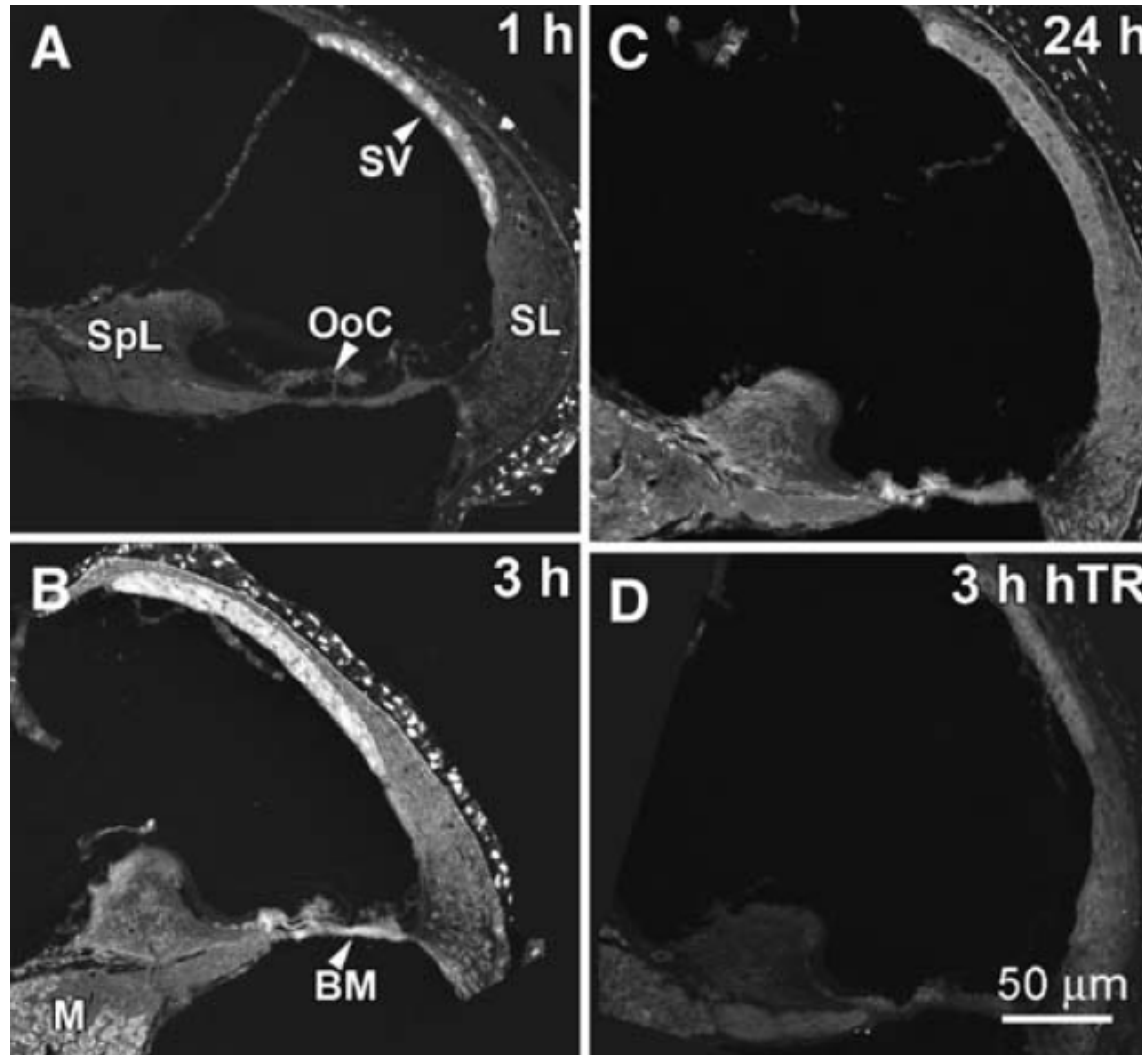




# GTTR enters zebrafish neuromast hair cells and is cytotoxic



## Cochlear uptake of GTTR in cryostat sections



# Reactive oxygen and nitrogen species<sup>a</sup> produced in the human body

## Reactive oxygen species<sup>b</sup>

### Radicals

Superoxide,  $O_2^{\cdot-}$

Hydroxyl,  $\cdot OH$

Peroxyl,  $RO_2\cdot$

Alkoxy,  $RO\cdot$

Hydroperoxyl,  $HO_2\cdot$

### Nonradicals

Hydrogen peroxide,  $H_2O_2$

Hypochlorous acid,  $HOCl$

Ozone,  $O_3$

Singlet oxygen,  $^1\Delta_g$

Hypobromous acid,  $HOBr$

## Reactive nitrogen species<sup>c</sup>

Nitric oxide,  $NO$

Nitrogen dioxide,  $NO_2$

Nitrous acid,  $HNO_2$

Nitrosyl cation,  $NO^+$

Nitroxyl anion,  $NO^-$

Dinitrogen tetroxide,  $N_2O_4$

Dinitrogen trioxide,  $N_2O_3$

Peroxynitrite,<sup>d</sup>  $ONOO^-$

Nitronium cation,  $NO_2^+$

Nitryl chloride,  $NO_2Cl$

Alkyl peroxynitrates,  $ROONO^-$

<sup>a</sup>These species have widely varying reactivities:  $\cdot OH$  reacts rapidly with almost everything, whereas  $H_2O_2$ ,  $O_2^{\cdot-}$ ,  $NO\cdot$  only react quickly with a few molecules.  $RO_2\cdot$ ,  $RO\cdot$ ,  $HOCl$ ,  $HOBr$ ,  $NO_2Cl$ ,  $NO_2\cdot$ ,  $ONOO^-$ , and  $O_3$  have intermediate reactivities.

<sup>b</sup>ROS is a collective term that includes both oxygen radicals and certain nonradicals that are oxidizing agents and/or are easily converted into radicals.

<sup>c</sup>RNS includes both nitrogen-containing radicals and nonradicals.

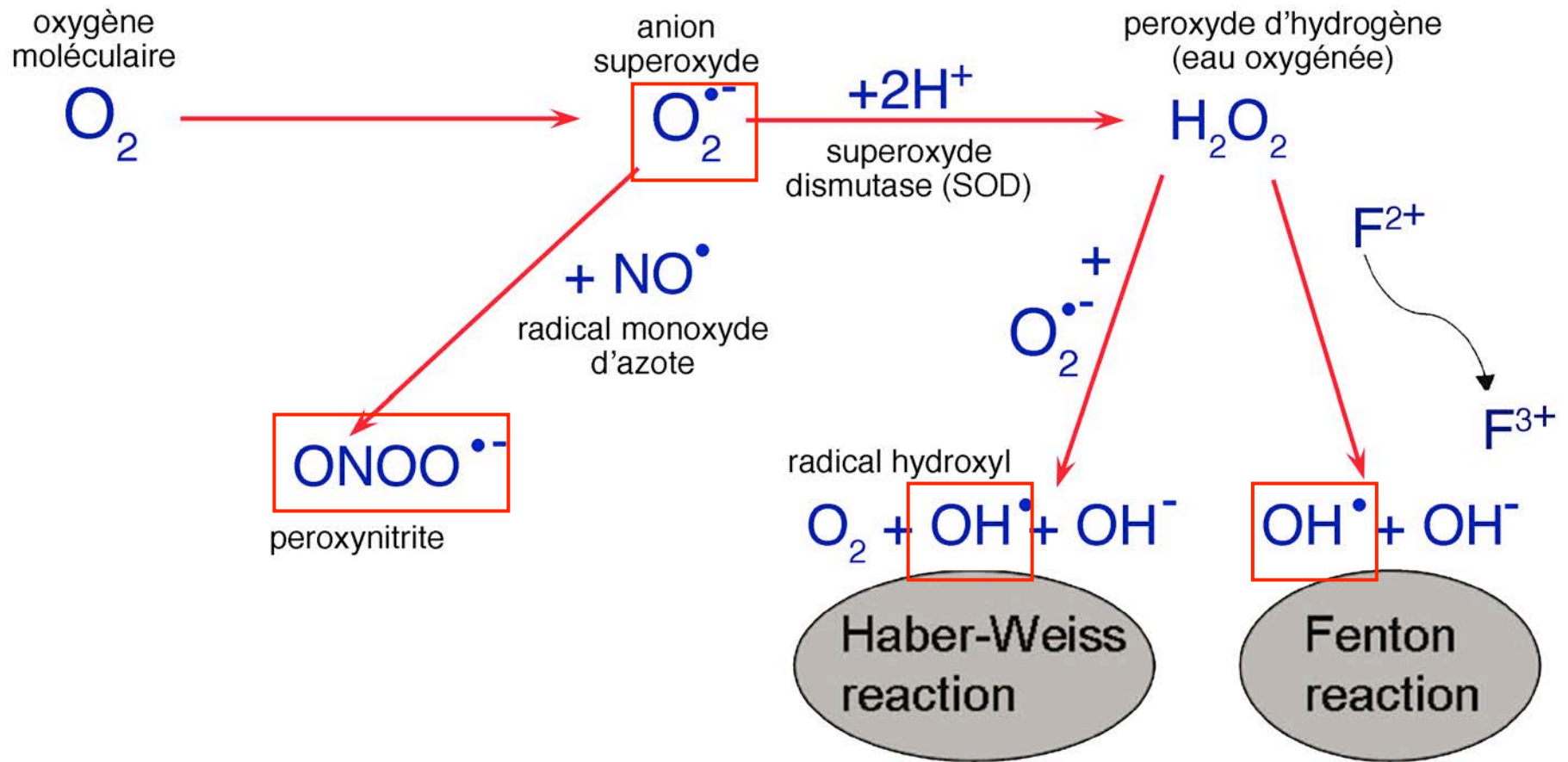
<sup>d</sup>Peroxynitrite can be regarded as an ROS or RNS.

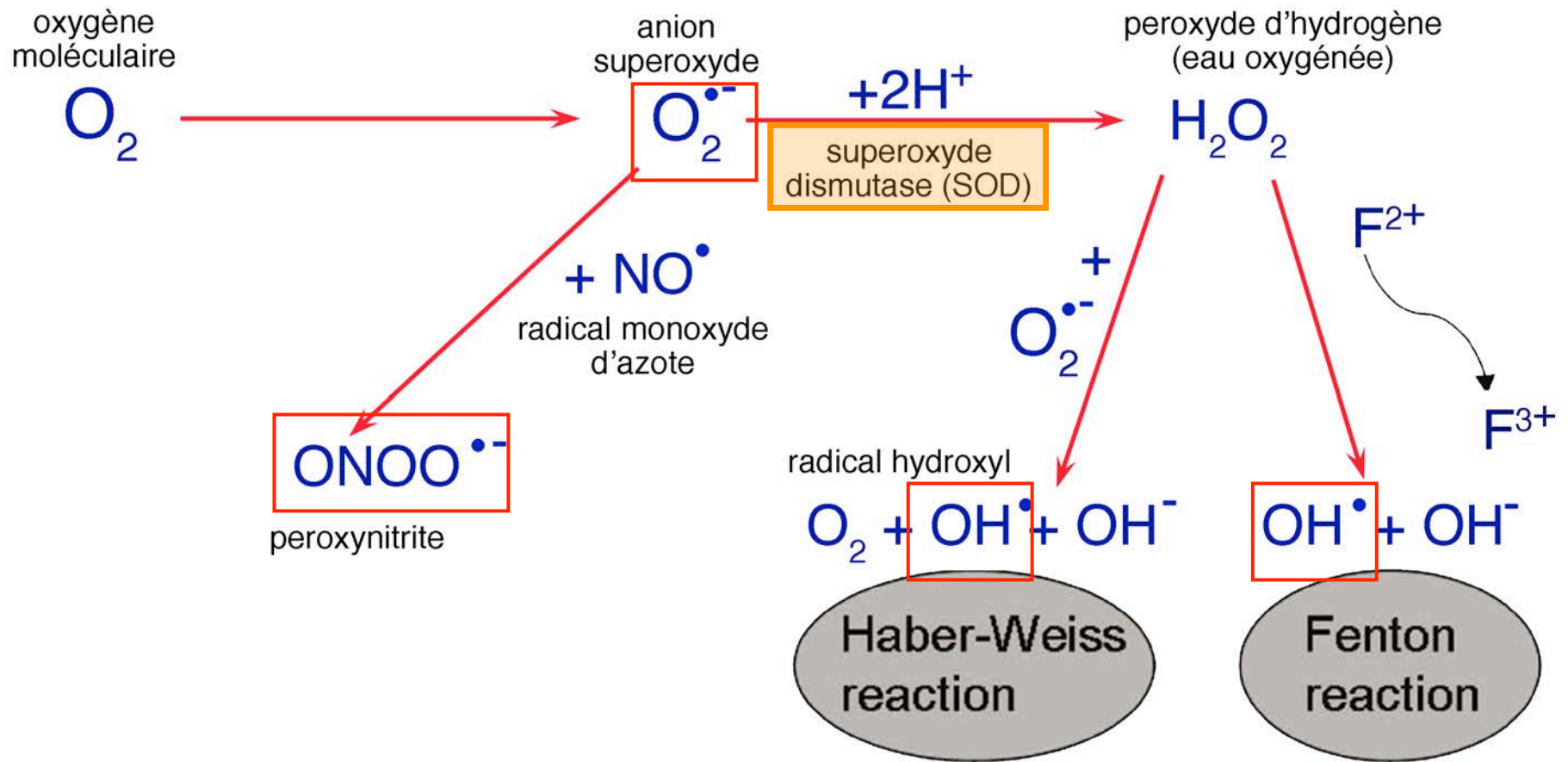
oxygène  
moléculaire



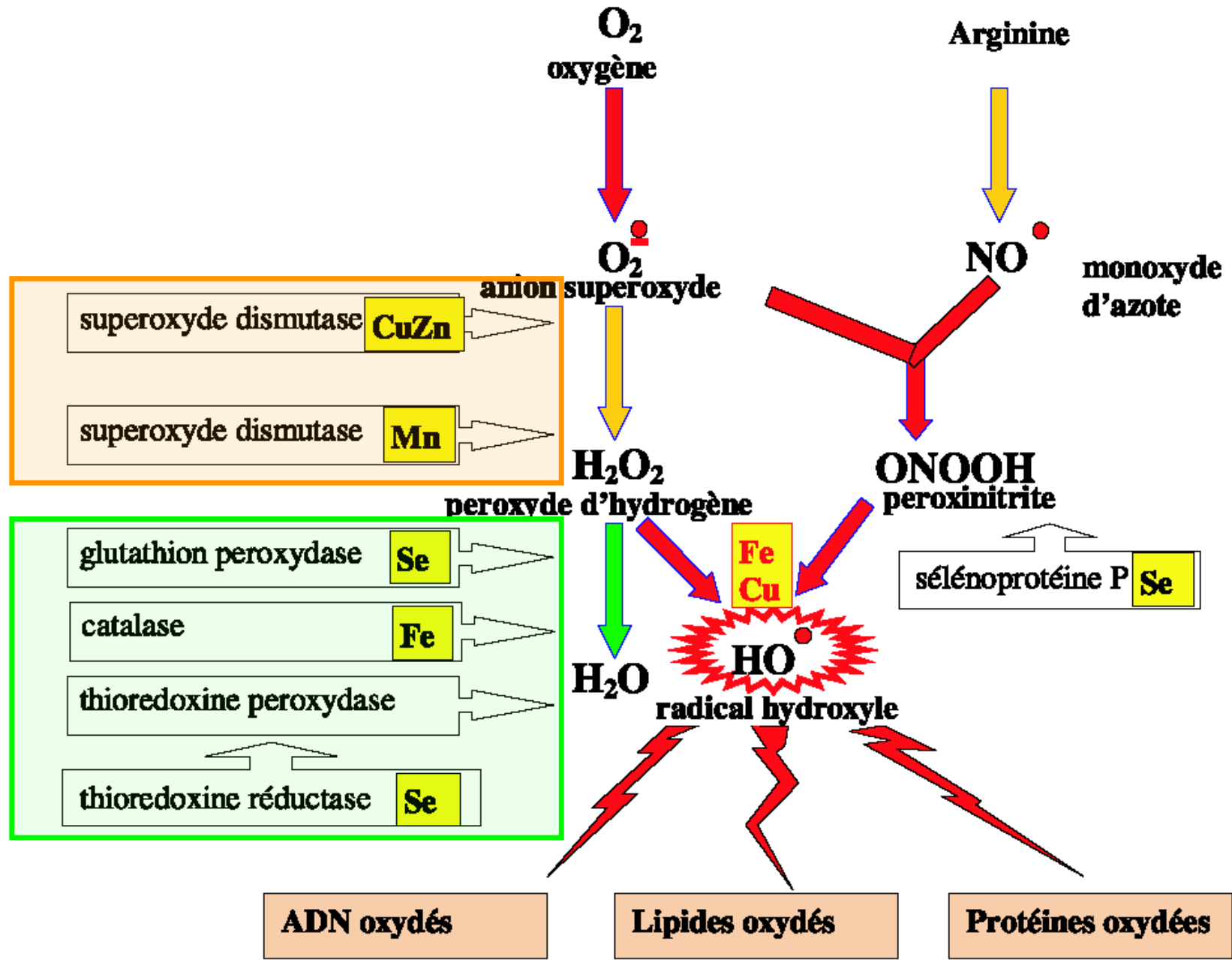
anion  
superoxyde



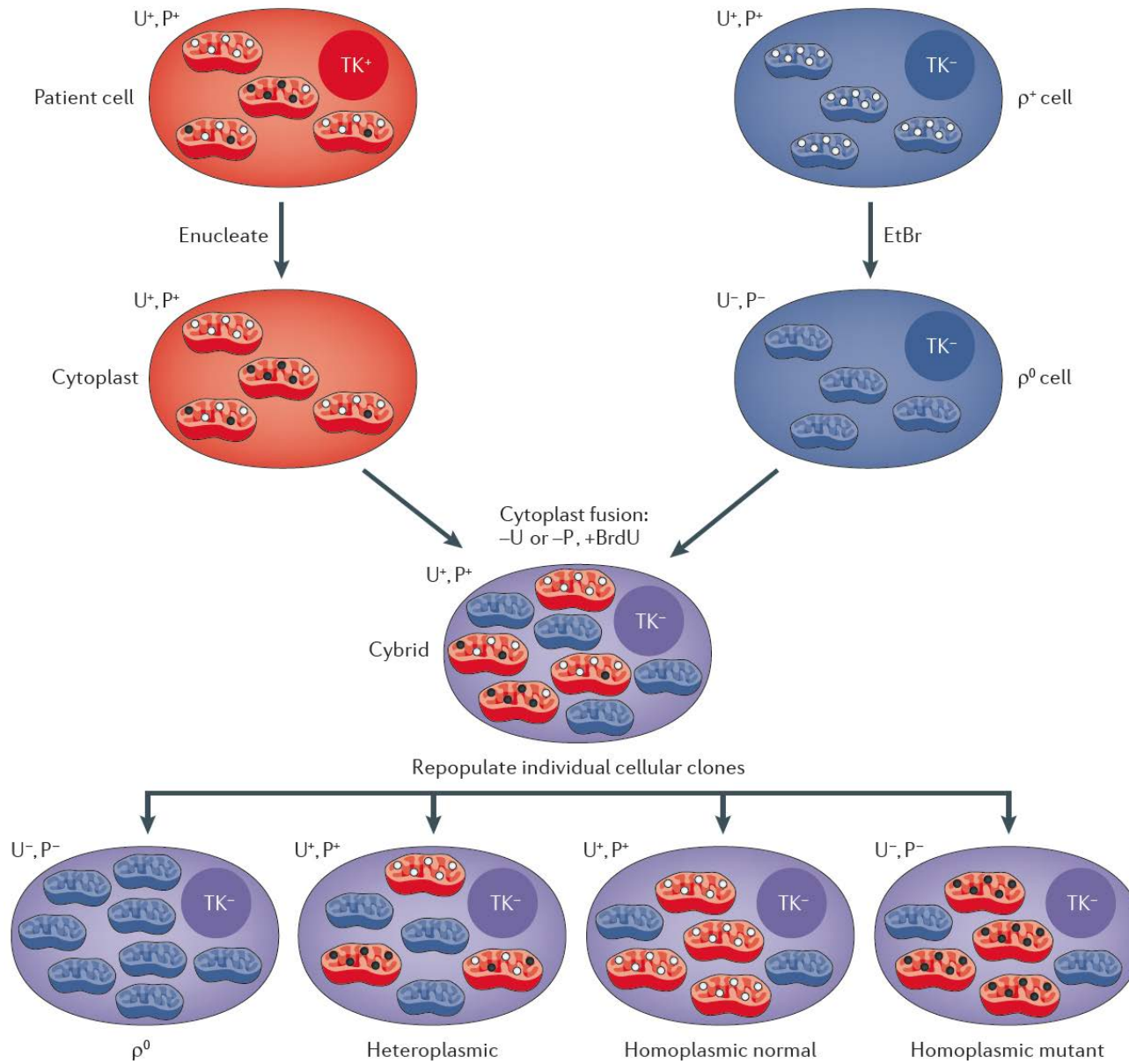








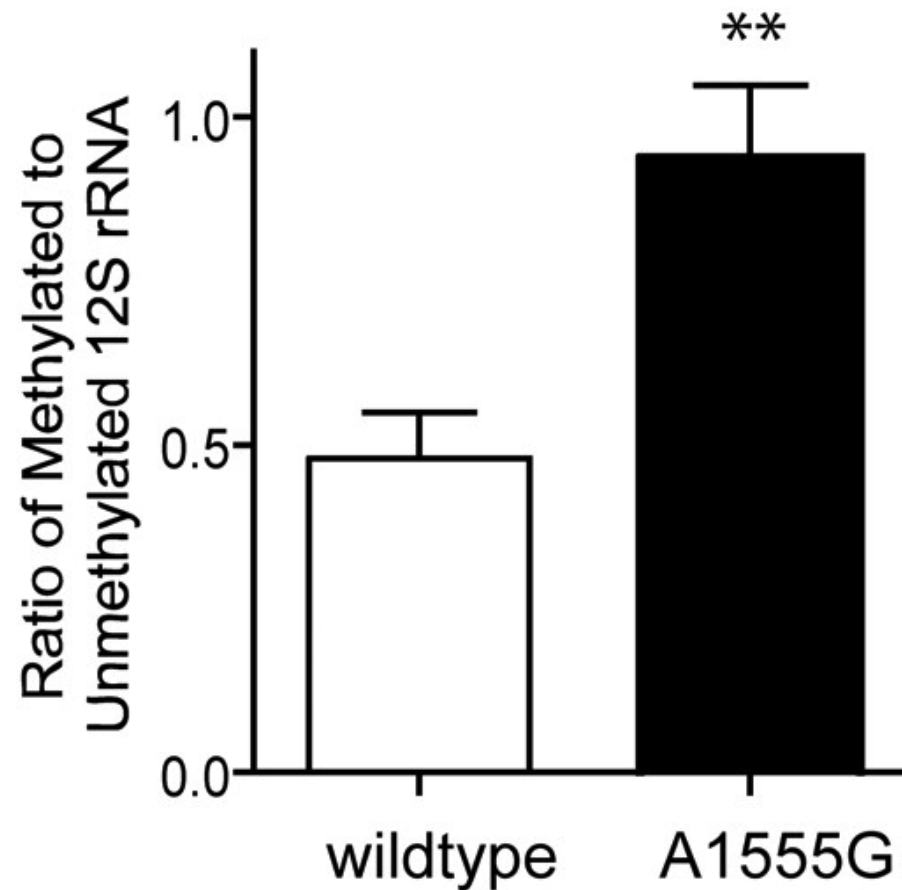
# Cybrides





Cells carrying the deafness-associated A1555G mtDNA mutation, like those that over-express h-mtTFB1, exhibit mitochondrial 12S hypermethylation

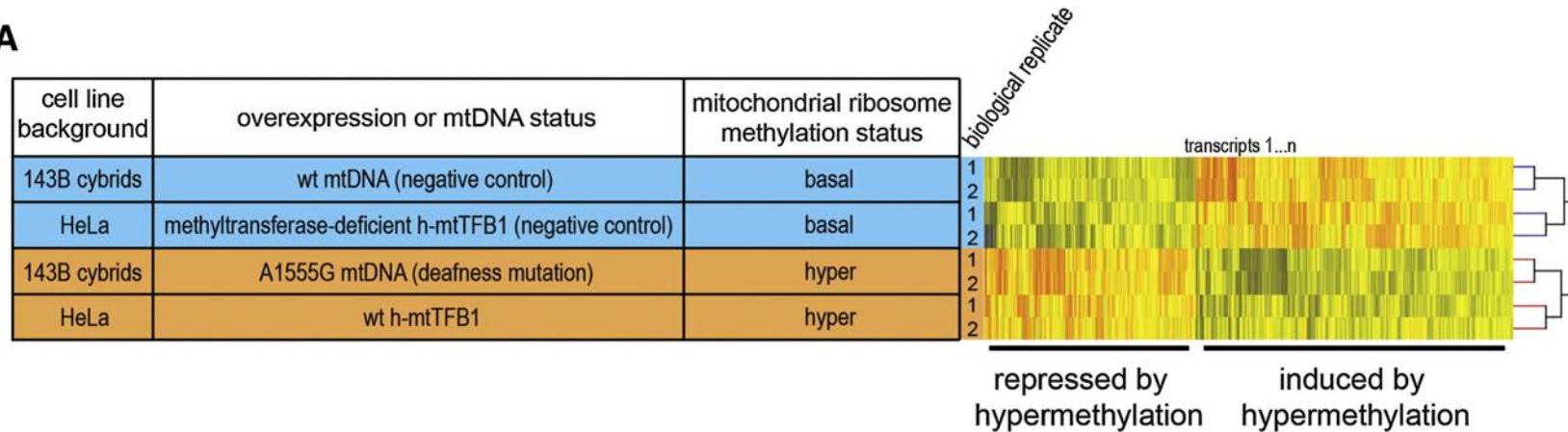
Les cybrides A1555G ont une hyperméthylation de l'ARN12S comme ceux qui hyperexpriment h-mtTFB1



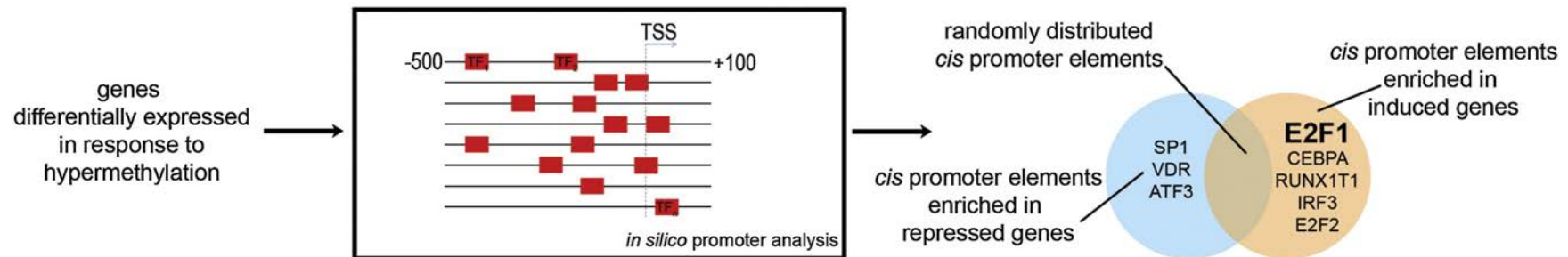
# Integrative genomic analysis reveals upregulation of nuclear transcription factor E2F1 in response to mitochondrial stress induced by mitochondrial ribosome hypermethylation

Les gènes dont la transcription est accrue dans les cybrides A1555G et dans les cybrids dont la concentration mitochondriale de TFB1 est augmentée possèdent dans leurs promoteurs des sites de fixation à E2F1

**A**

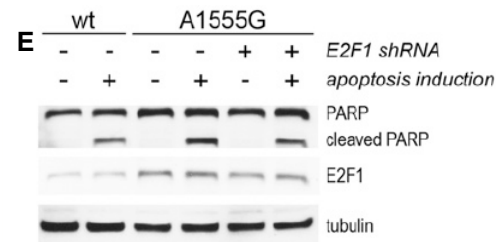
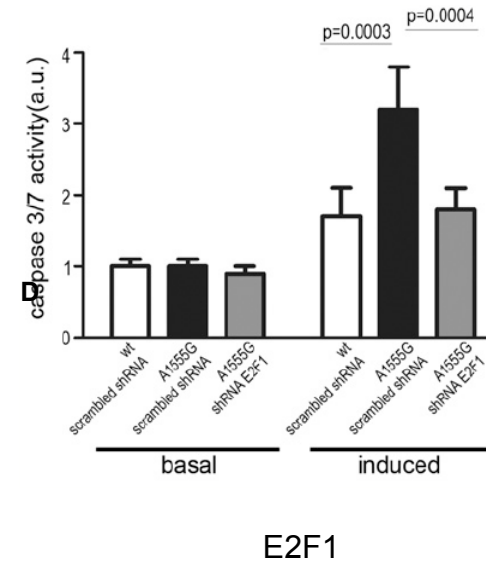
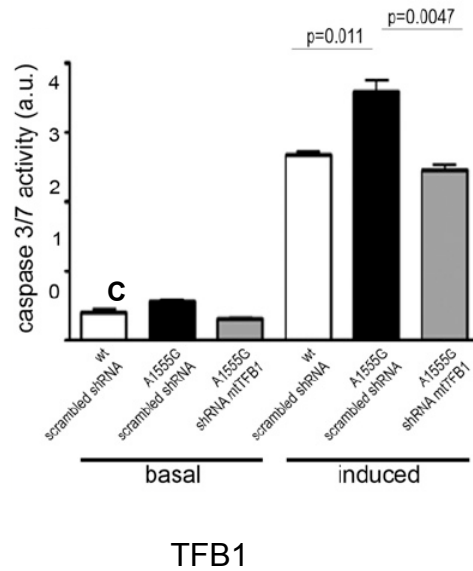


**B**



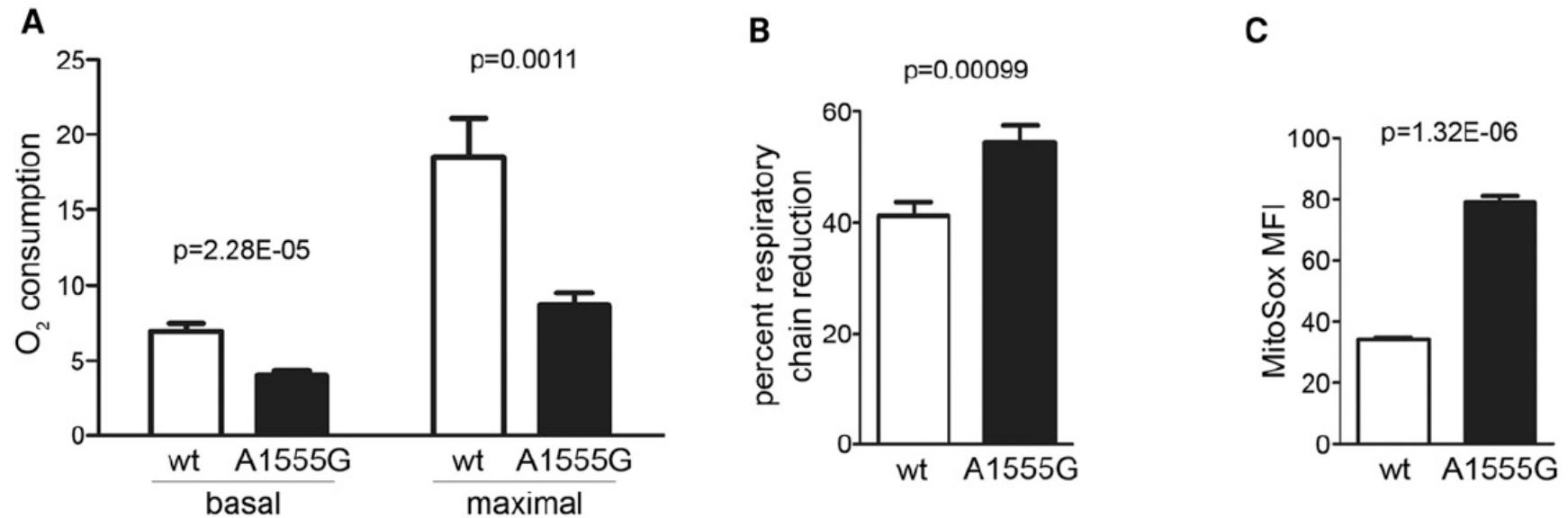
# Hypermethylation of mitochondrial 12S rRNA leads to activation of E2F1- and E2F1-dependent apoptosis

Cybrides A1555G +/- aminoglycosides: rôle de TFB1 et E2F1 dans l'apoptose induite et non-induite



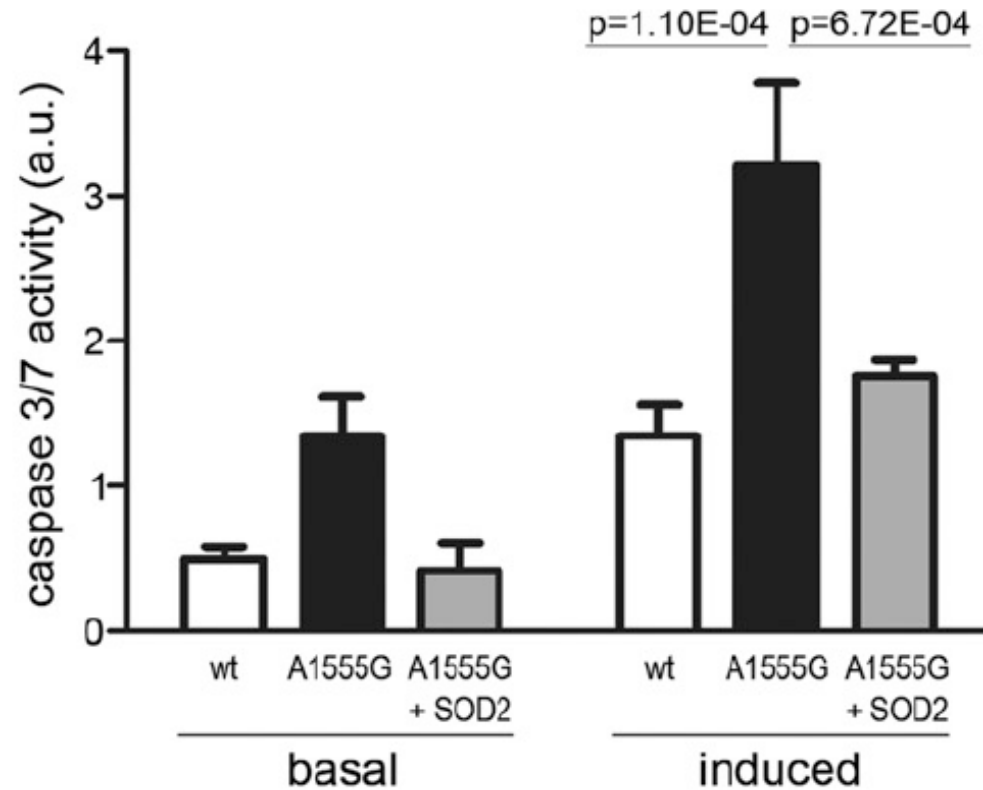
A proapoptotic mitochondrial stress-signaling pathway that involves mitochondrial ROS-dependent activation of E2F1 by AMPK

Cybrides A1555G : production de ROS accrue



A proapoptotic mitochondrial stress-signaling pathway that involves mitochondrial ROS-dependent activation of E2F1 by AMPK

Cybrides A1555G +SOD2 : diminution (des ROS) de l'apoptose



mutation mitochondriale A1555G,



hyperméthylation de l' ARN 12S de la petite sous-unité  
du ribosome mitochondriale,



perturbation de la biogenèse mitochondriale,  
augmentation des ROS,

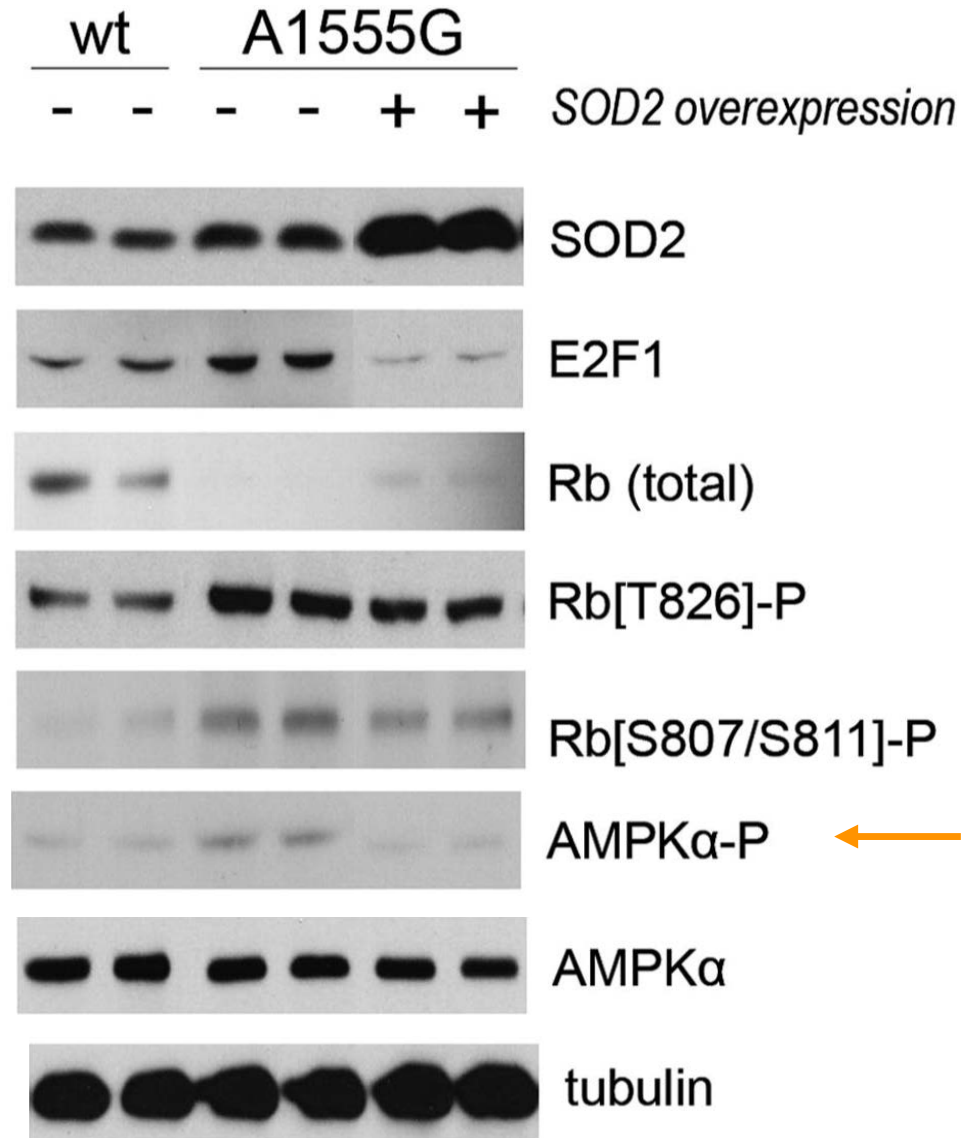


augmentation de l' expression de E2F1



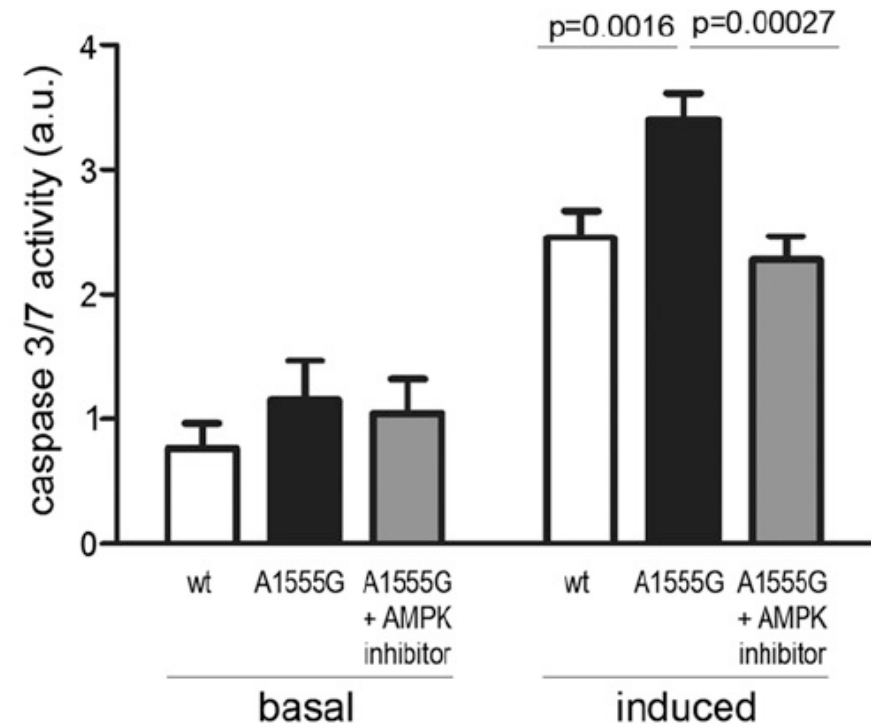
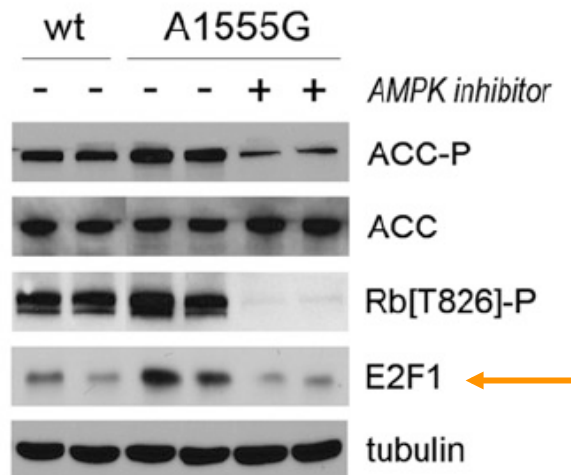
apoptose cellulaire

A proapoptotic mitochondrial stress-signaling pathway that involves mitochondrial ROS-dependent activation of E2F1 by AMPK



A proapoptotic mitochondrial stress-signaling pathway that involves mitochondrial ROS-dependent activation of E2F1 by AMPK

Cybrides A1555G +inhibiteur de l'AMPK : diminution de l'apoptose





mutation mitochondriale A1555G,



hyperméthylation de l'ARN 12S de la petite sous-unité  
du ribosome mitochondriale,



perturbation de la biogenèse mitochondriale,  
augmentation des ROS,



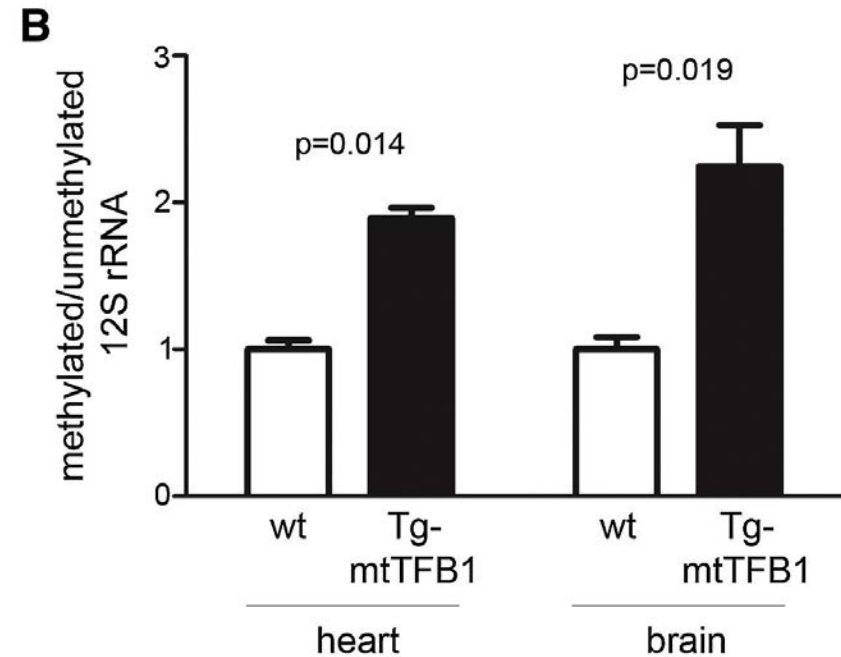
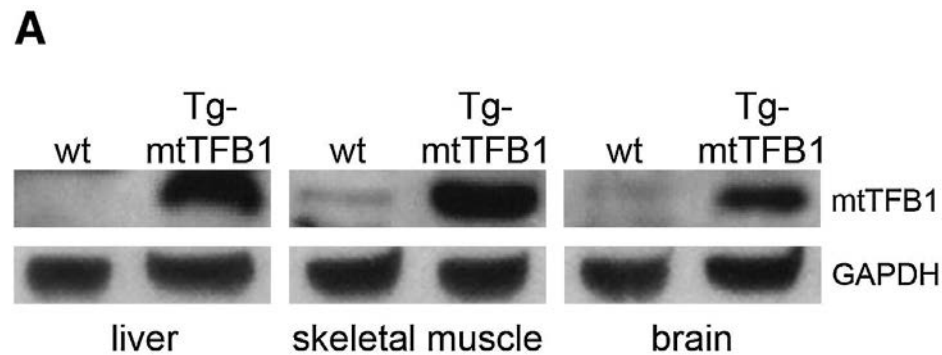
activation de l'AMPK  
augmentation de l'expression de E2F1  
et activation de E2F1



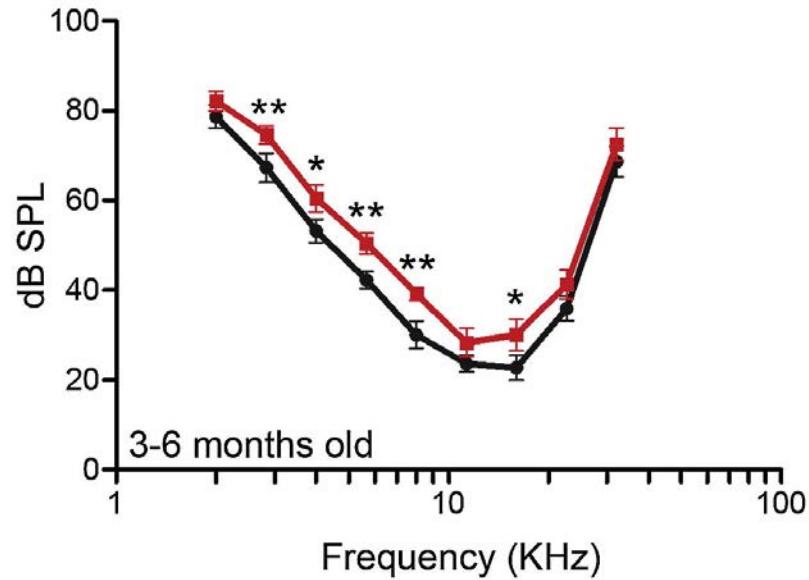
apoptose cellulaire

Transgenic mice that overexpress the mtTFB1 rRNA methyltransferase exhibit 12S hypermethylation and progressive hearing loss

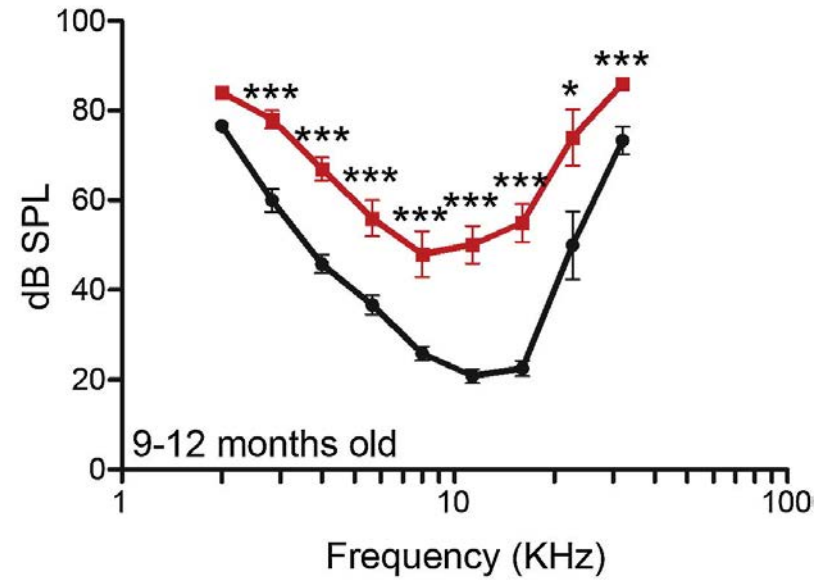
Souris transgeniques qui hyperexpriment mTFB1



# Transgenic mice that overexpress the mtTFB1 rRNA methyltransferase exhibit 12S hypermethylation and progressive hearing loss

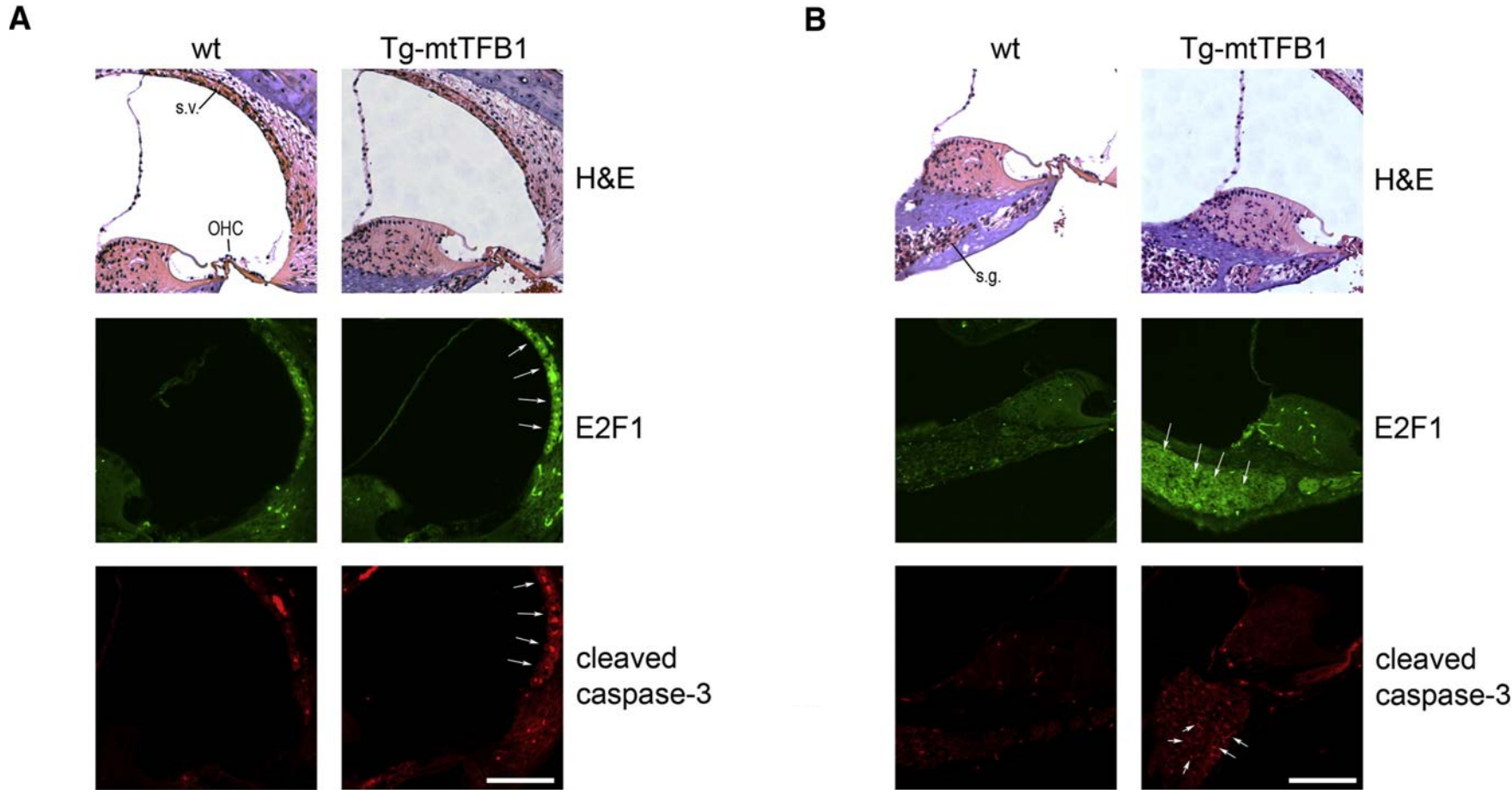


—●— wt  
—■— Tg-mtTFB1



—●— wt  
—■— Tg-mtTFB1

Deafness pathology in Tg-mtTFB1 mice involves upregulation of E2F1 and apoptosis in the stria vascularis and spiral ganglion neurons of the inner ear



Deafness pathology in Tg-mtTFB1 mice involves upregulation of E2F1 and apoptosis in the stria vascularis and spiral ganglion neurons of the inner ear

