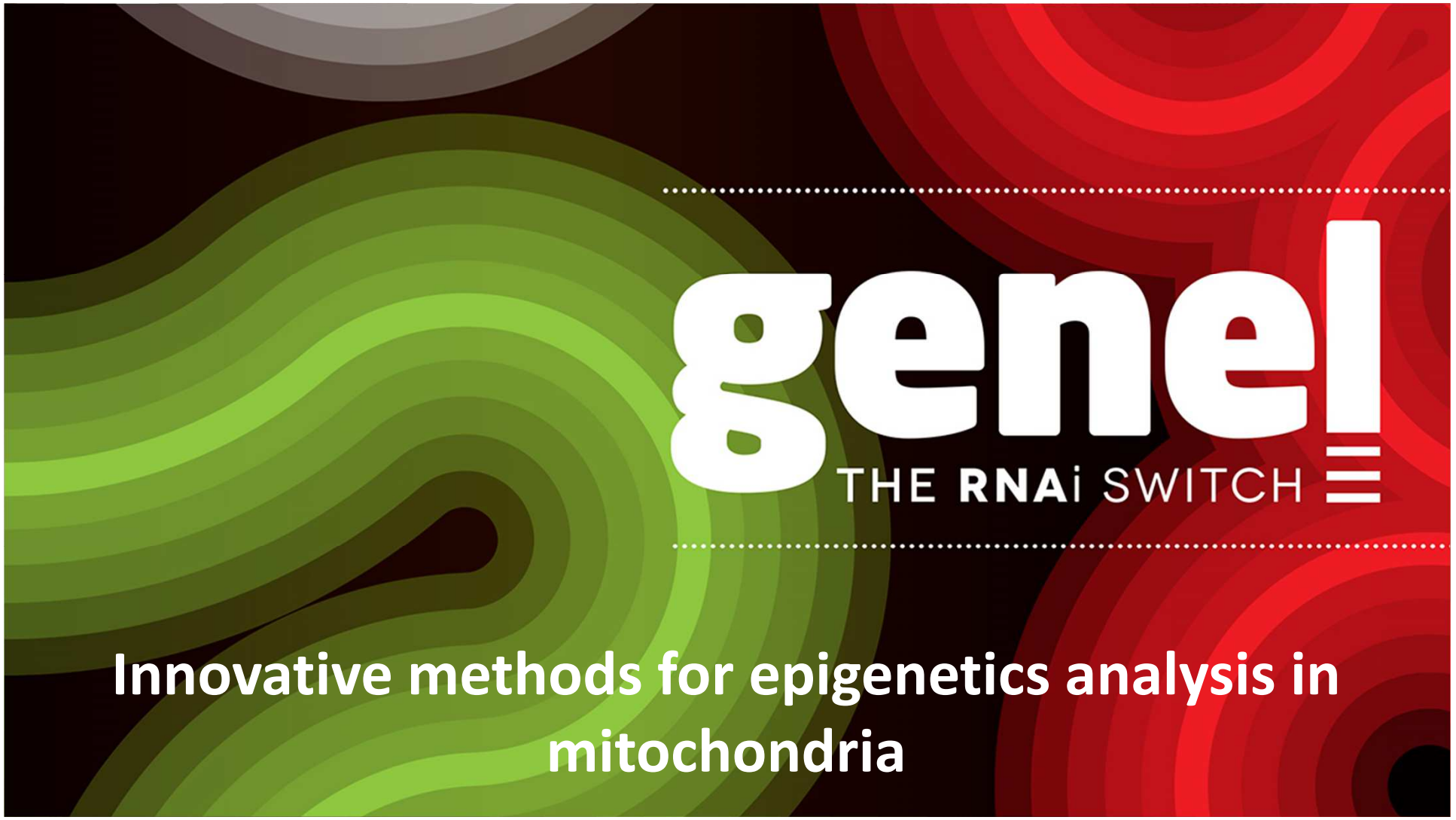
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Innovative methods for epigenetics analysis in mitochondria

SOMMAIRE

- **Epigenetic in nuclei and mitochondria**
- **Innovative methods for microRNAs study**
  Identification for the first time : microRNAs in mitochondria
- **Biological breakthroughs using these new methods**
- **Conclusions**

Epigenetic in nuclei and mitochondria

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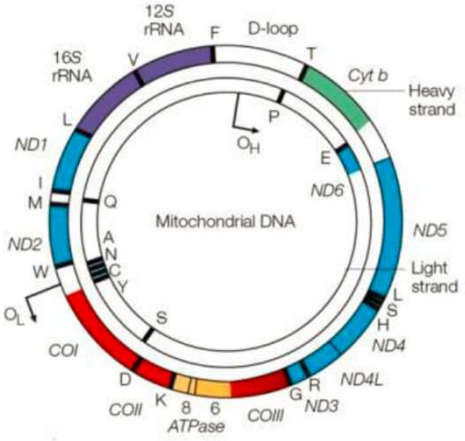
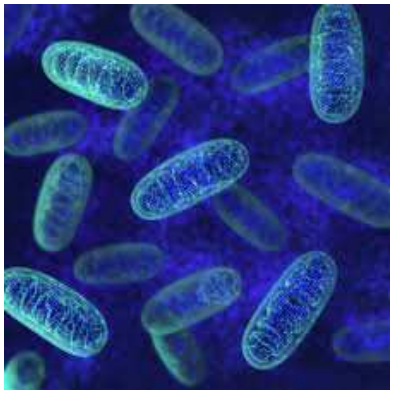
Mitochondria



- mtDNA : extranuclear genome came from your

Mother -37 genes : 13 for proteins (phosphorilation enzymes) [N.B., all other proteins coded in nuclear DNA]; 22 for tRNAs; 2 for rRNAs (12S, 16S)

- Hundreds to thousands mitochondria per cell
- 2-10 mtDNA copies per mitochodrion



Functions :

-energy production : 95% of cellular ATP

-role in apoptosis

-…

Stimpfel et al, stem cell rev and rep

Mitochondria : frontline of cellular response to environment

- mtDNA defects or mitochondrial dysfunction induce degenerative diseases, ageing

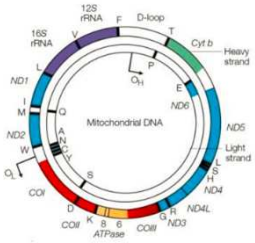
- mtDNA is targeted by ultraviolet radiation.

It maybe a candidate biomarker of cumulative exposure in skin

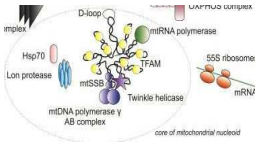
- Oxidative damage 5 to 10 times higher than nuclear DNA (direct exposure to endogenous ROS, lacks protective histones, diminished DNA repair capacity)

The main epigenetic mechanism

Mitochondrion



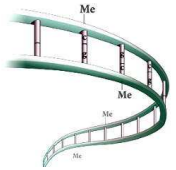
mtDNA methylation



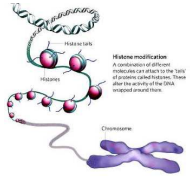
nucleoid modification

Nucleus

DNA methylation



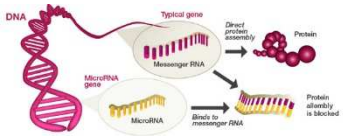
histone modification



Mitochondria non coding RNAs : microRNAs

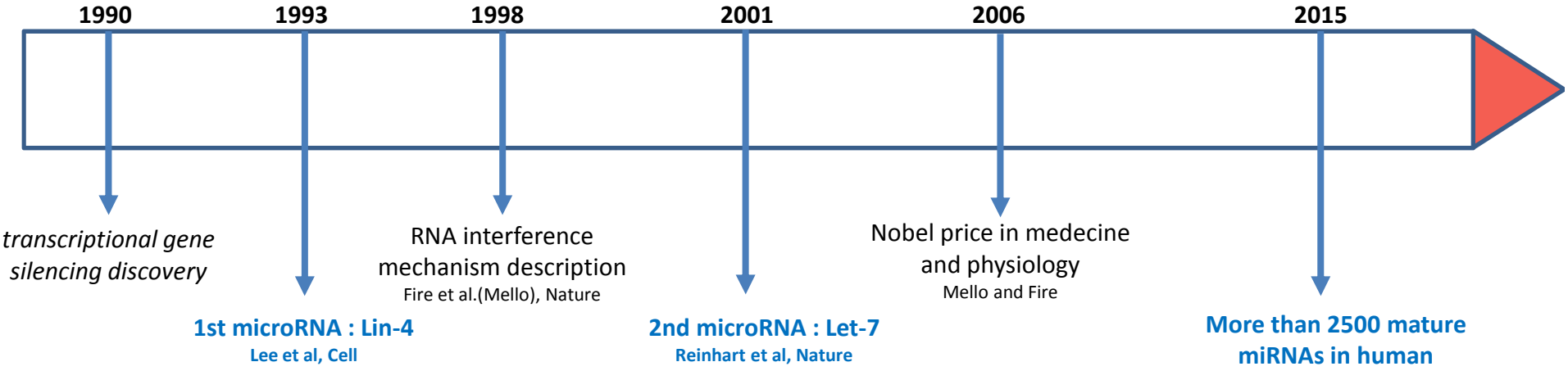
Needs for news dedicated methods

Nuclear non coding RNAs : microRNAs



Stimpfel et al, stem cell rev and rep

RNA interference a breakthrough in gene regulation



One microRNA regulates several genes (near from 1000 targets)

One gene can be regulated by several microRNAs

Study function of microRNAs are becoming increasingly complex

MicroRNA role in mitochondria?

Innovative methods for microRNAs analysis in mitochondria

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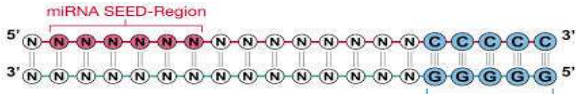
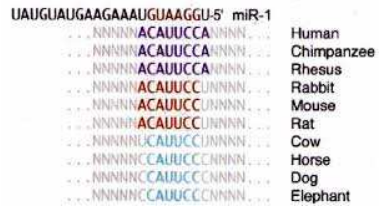
In silico technologies for microRNA studies

Seed sequence



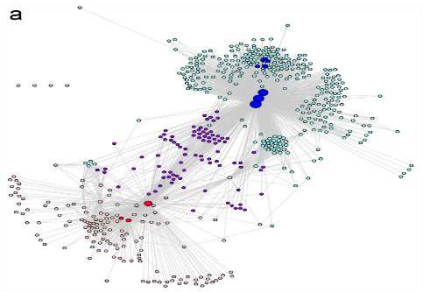
Evolutionary conservation



Databases

Source image : http://www.b2bnn.com/

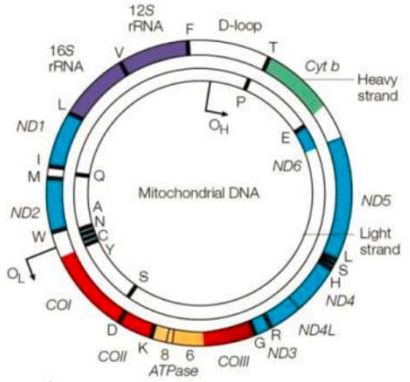
-Index all microRNAs (seed sequence and conservation)
-Predict microRNAs biological gene targets
-Predict molecular function of microRNAs



In silico analysis, a guide for the direction we should take

miR Databases

mtDNA sequence



alignment

```
mtDNA
 UUAAUUCCCCUAAAAAUCUUUGAAAUAGGGCCCGUAUUUACCCUAUAGCACCCCCUCUAC
   |||| ||||||||||||  ||
1 UAAUGCCCCUAAAAAUCCUUAU  22
hsa-miR-365  (evalue: 0.045)
```

Barrey and Saint-Auret et al, Plos one

miRNA ID	Accession #	Strand	Score	Evalue
hsa-mir-1267	MI0006404	–	145.6	0.022
hsa-mir-302a	MI0000738	+	143	0.035
hsa-let-7b	MI0000063	–	138	0.055
hsa-mir-1296	MI0003780	+	135.9	0.065
hsa-mir-522	MI0003177	–	131.1	0.13
hsa-mir-7-2	MI0000264	+	128.5	0.14
hsa-mir-632	MI0003647	+	126.7	0.21
hsa-mir-548k	MI0006354	+	122.2	0.3
hsa-mir-541	MI0005539	+	124.4	0.31
hsa-mir-1256	MI0006390	+	121.4	0.32
hsa-mir-576	MI0003583	–	121.6	0.39
hsa-mir-412	MI0002464	+	121.8	0.4
hsa-mir-1273	MI0006409	–	119.7	0.46
hsa-mir-320a	MI0000542	–	121.4	0.47
hsa-mir-595	MI0003607	–	120.1	0.48
hsa-mir-1275	MI0006415	–	120.8	0.52
hsa-mir-526b	MI0003150	+	119.3	0.61
hsa-mir-320b-1	MI0003776	+	118.6	0.69
hsa-mir-1183	MI0006276	+	117.7	0.7
hsa-mir-1243	MI0006373	–	117	0.73
hsa-mir-548d-2	MI0003671	+	116.3	0.77
hsa-mir-1322	MI0006653	+	118.6	0.78
hsa-mir-329-1	MI0001725	+	117.4	0.8
hsa-mir-329-2	MI0001726	+	117	0.81
hsa-mir-548f-5	MI0006378	–	116.8	0.81
hsa-mir-518f	MI0003154	–	115.9	0.89
hsa-mir-1286	MI0006348	+	116.8	0.9
hsa-mir-365-2	MI0000769	+	114	0.9
hsa-mir-579	MI0003586	–	114.8	0.91
hsa-mir-26a-2	MI0000750	+	116	0.92
hsa-mir-548a-2	MI0003598	–	114.5	0.96
hsa-mir-532	MI0003205	–	114.6	1
hsa-mir-889	MI0005540	–	115.7	1

33 pre-miR

HIS : hsa-mir-302a

Hsa-mir-let7b

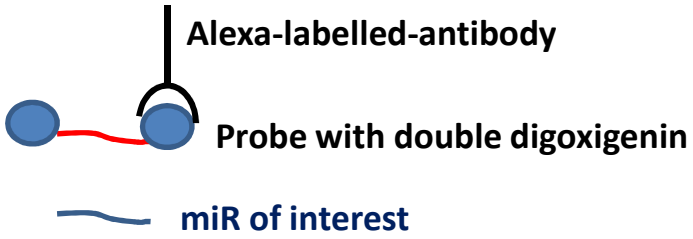
miRNA ID	Accession #	Strand	Score	Evalue
hsa-miR-365	MI0000767	+	137	0.045
hsa-miR-31*	MI0000089	–	133.8	0.068
hsa-miR-652	MI0003667	+	129.6	0.12
hsa-miR-557	MI0003563	+	125.4	0.19
hsa-miR-590-5p	MI0003602	+	124.3	0.23
hsa-miR-7-2*	MI0000264	+	123	0.27
hsa-miR-516b	MI0003167	+	122.4	0.29
hsa-miR-765	MI0005116	–	121.4	0.35
hsa-miR-127-5p	MI0000472	+	121.1	0.35
hsa-miR-190b	MI0005545	–	121.1	0.37
hsa-miR-637	MI0003652	+	120.6	0.34
hsa-miR-936	MI0005758	+	118.1	0.51
hsa-miR-582-3p	MI0003589	–	117.7	0.54
hsa-miR-451	MI0001729	+	117.5	0.55
hsa-miR-606	MI0003619	+	117.5	0.58
hsa-miR-198	MI0000240	–	117	0.59
hsa-miR-328	MI0000804	+	115.5	0.72
hsa-miR-132*	MI0000449	+	115	0.76
hsa-miR-186	MI0000483	–	114	0.86
hsa-miR-10b*	MI0000267	+	113.7	0.9
hsa-miR-197	MI0000239	+	112.9	0.99
hsa-miR-589*	MI0003599	–	112.9	0.91
hsa-miR-556-3p	MI0003562	–	112.8	1
hsa-miR-135a	MI0000452	–	112.4	1
hsa-miR-582-5p	MI0003589	+	112.4	1

25 miR

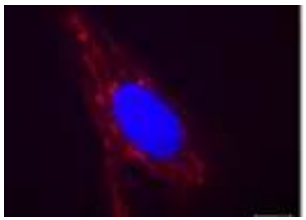
HIS : hsa-mir-365

Barrey and Saint-Auret et al, Plos one

Colocalisation procedure



Mitochondria labelling by mitotracker



Controls for specific labelling

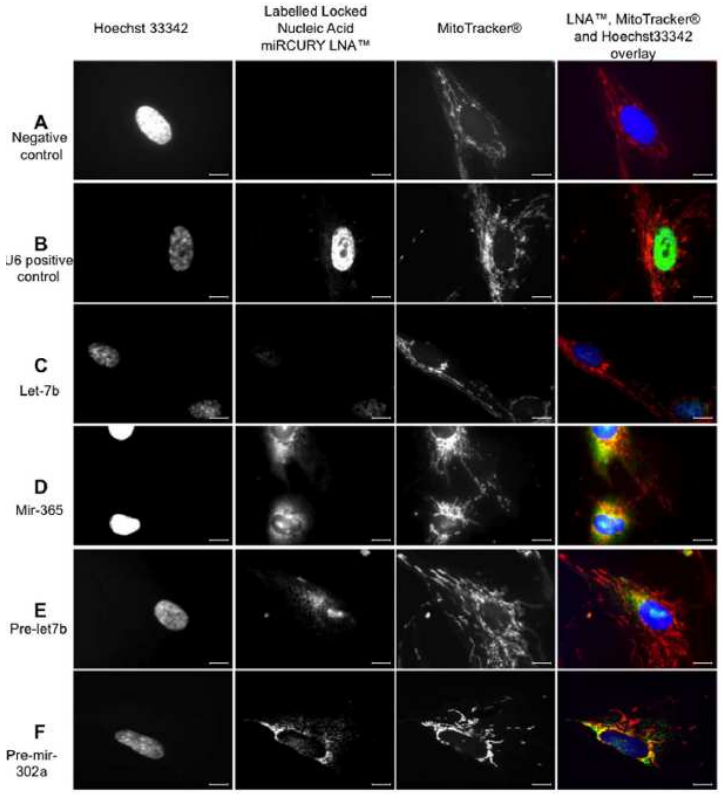
-U6 nuclear small RNA only in nuclear
-Double digoxigenin Scramble mir
-Competition with probe without digoxigenin

Confocal microscopy and quantification

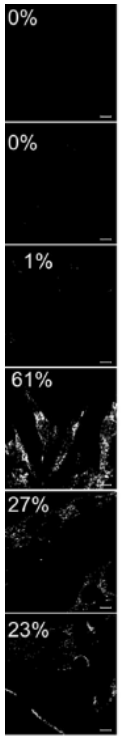
Barrey and Saint-Auret et al, Plos one

Results

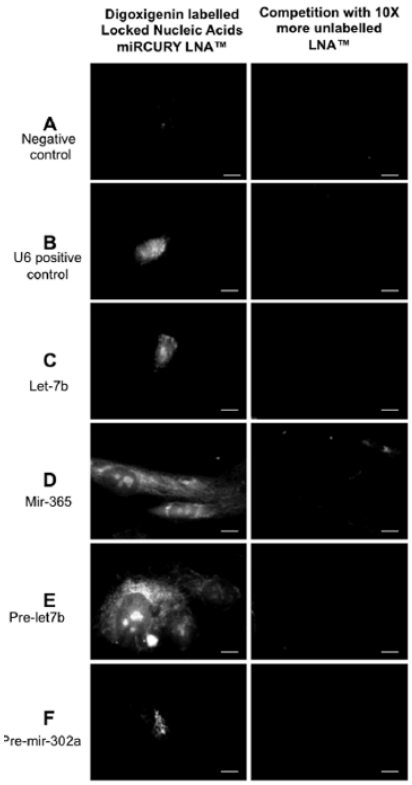
MicroRNA and mitochondria localisation



Confocal anlysis for co-localisation



Competition test



Controls : Specific labelling

-U6 nuclear small RNA only in nuclear

-double digoxigenin

Scramble mir : no signal

-Efficient Competition

Results :

-hsa-pre-mir-let7

-hsa-pre-mir-302a

-hsa-mir-365

Colocalisation with mitochondria

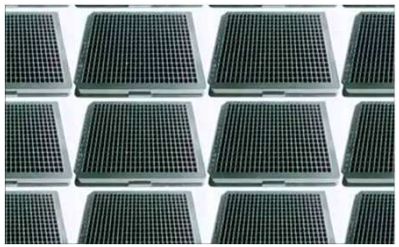
Barrey and Saint-Auret et al, Plos one

MicroRNAs Quantification

Mitochondria purification
RNAse treatment

→ **Purity validation**

Dedicated Mirome analysis :



→ **Q-RT-PCR**
RNA seq

46 microRNAs identified in pure mitochondrial extract including miR of interest

Biological breakthroughs using new methods

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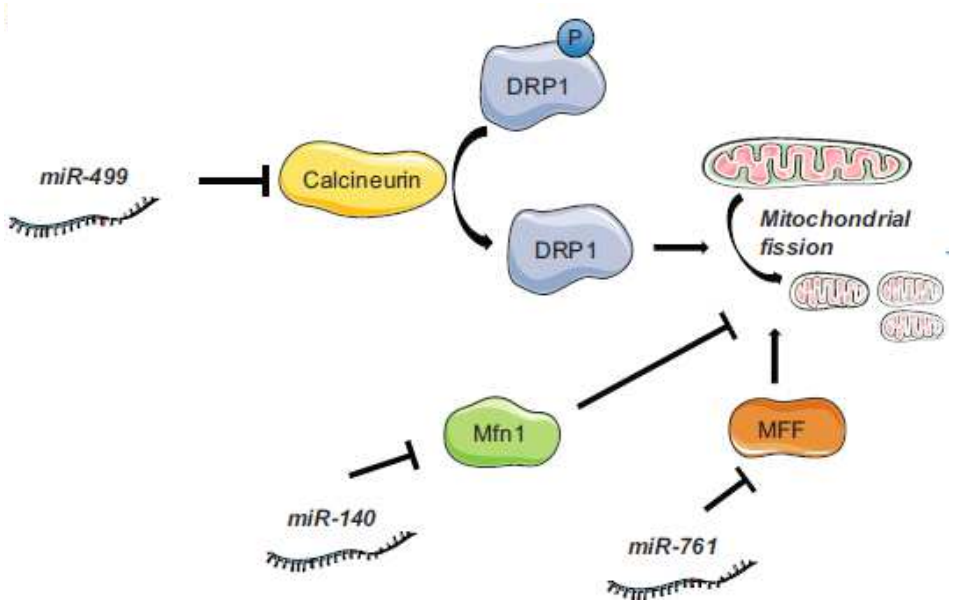
MicroRNAs functions in Mitochondria

miRNA	Target	miRNA Location	Model	Reference
miR-338	COXIV	Cytosol	Sympathetic neurons, rat	16
miR-23a/b	Glutaminase	Cytosol	Lymphoma cells, human	17
miR-210	COXX; iron-sulfur cluster homolog	Cytosol	Colon epithelial cells, human	18
miR-15a	Uncoupling protein-2	Cytosol	Pancreatic β cells, mouse	19
miR-126	Insulin receptor substrate-1	Cytosol	Breast cancer cells, human	20
miR-696	Peroxisome proliferator-activated receptor-γ coactivator 1-α	Cytosol	Skeletal myocytes, mouse	21
miR-743a	Malate dehydrogenase	Cytosol	Brain neurons, mouse	22
miR-17*	Mitochondrial antioxidant enzymes	Cytosol	Cancer cells, human	23
miR-130a	COXIII†	Mitochondria	Liver, rat	9
miR-181c	COXI	Mitochondria	Cardiomyocytes, rat	13

- From cytosol and regulate mtRNA
- From cyotosol and regulate nRNA
- From mtDNA and regulate mtRNA
- From mtDNA and regulate nRNA

Latronico et al , circulation research

MiR in Mitochondria dynamics (autophagy)



miRNA	Target	Effect on autophagy	Reference
miR-101	STMN1, RAB5A and ATG4D	Inhibitor	[70]
miR-204	LC3	Inhibitor	[71]
miR-30a	Beclin 1	Inhibitor	[72,76]
miR-137	NIX, FUNDC1	Inhibitor (mitophagy)	[77]

Filipe V. Duarte et al , genes

Conclusions

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- **Epigenetic regulates a large majority of biological process in nucleus and mitochondrion. We demonstrated for the first time the presence of miR in mitochondria using new methods**
  - **In silico analysis, a guide for the direction we should take**
  - **HIS a new method to localize and colocalise microRNA in cells.**

- **Outcomes : study of others organites and others cellular processes**

THANK YOU

Learn more…

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