



Looking for a mitochondrial role of telomerase reverse transcriptase

Tutor: Prof.ssa Antonella Sgura
Co-tutor: Dr. Ion Udroi

Erica Rossi

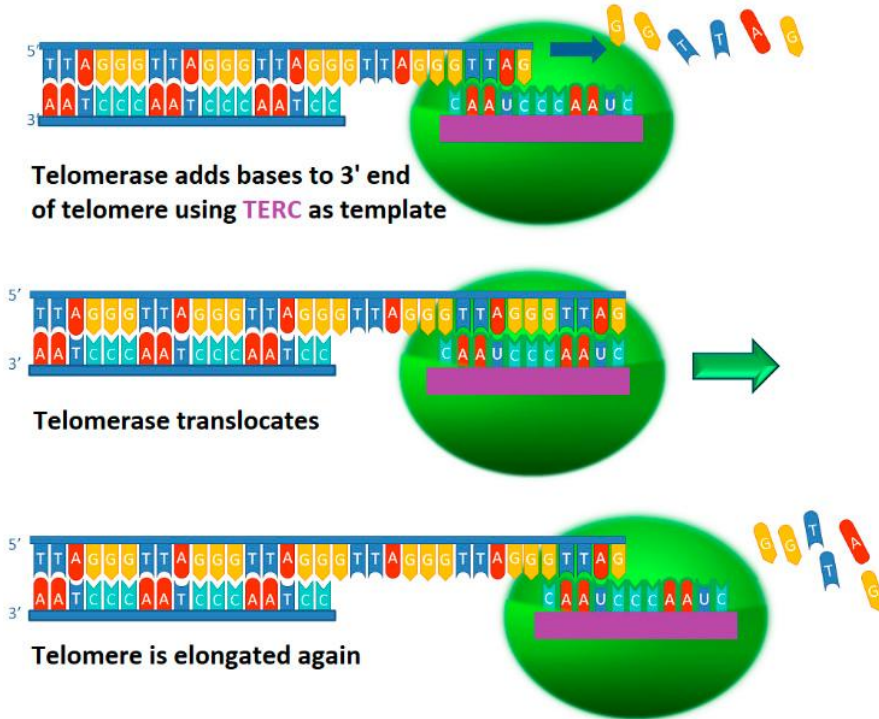
INTRODUCTION

TERT PROTEIN

Canonical function

Extra-telomeric roles

Telomere DNA elongation



Transcriptional regulator

NF- κ B Pathway
Wnt/ β -catenin
Pathway

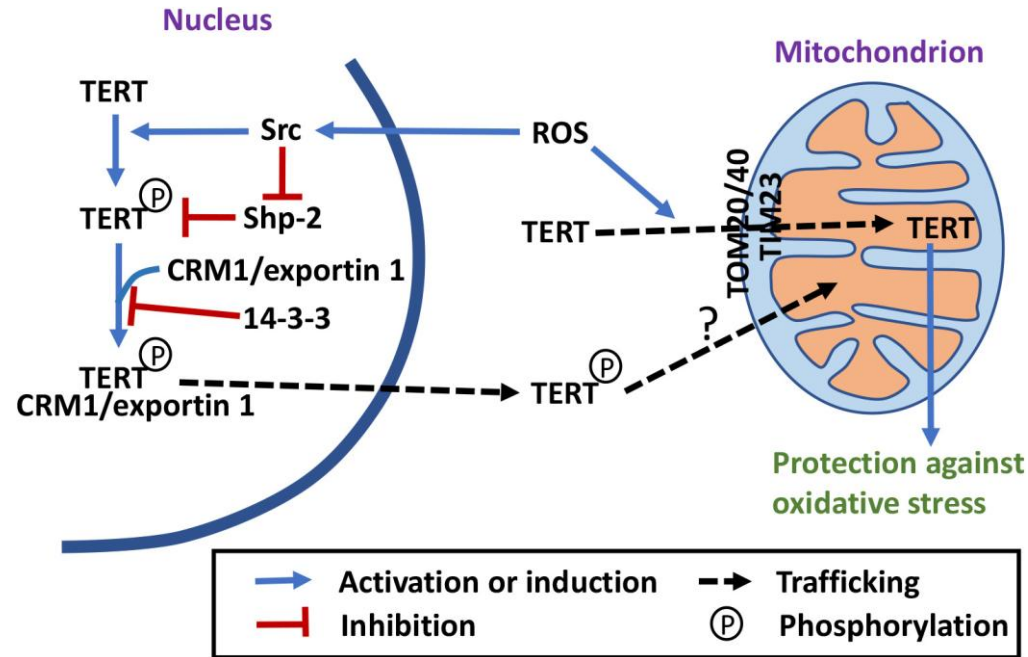
Regulator of
cellular
functionality

Anti-apoptotic
Anti-oxidant

Mitochondrial
role

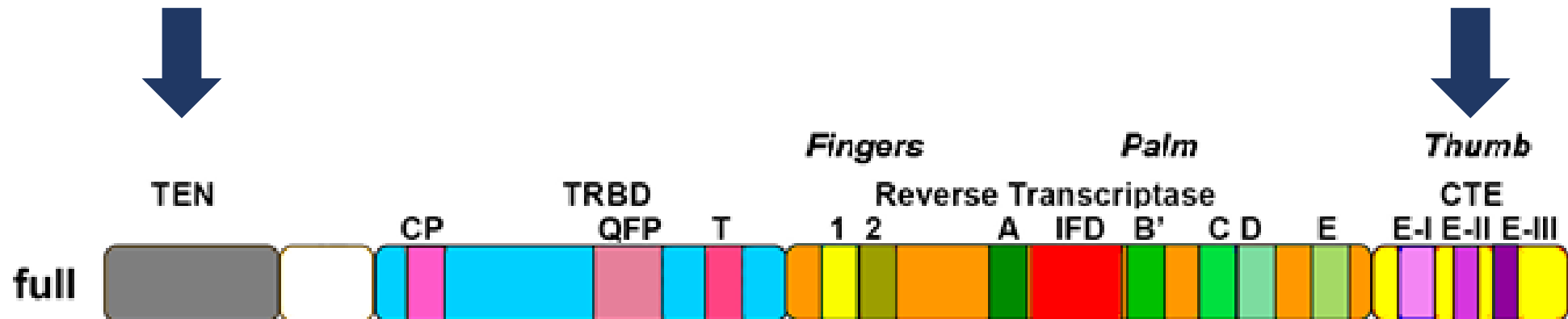
INTRODUCTION

TERT IN MITOCHONDRIA



Mitochondrial Targeting Sequence, MTS

Nuclear Export Signal, NES



INTRODUCTION

TERT IN MITOCHONDRIA

Article

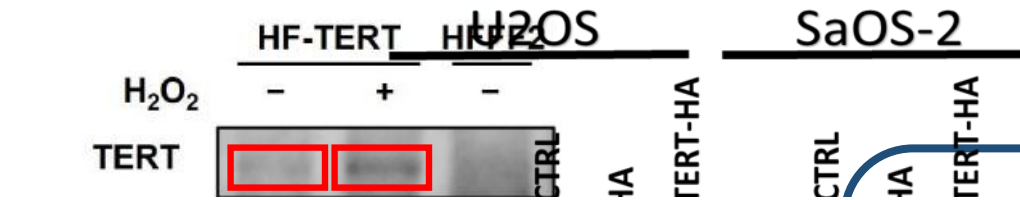
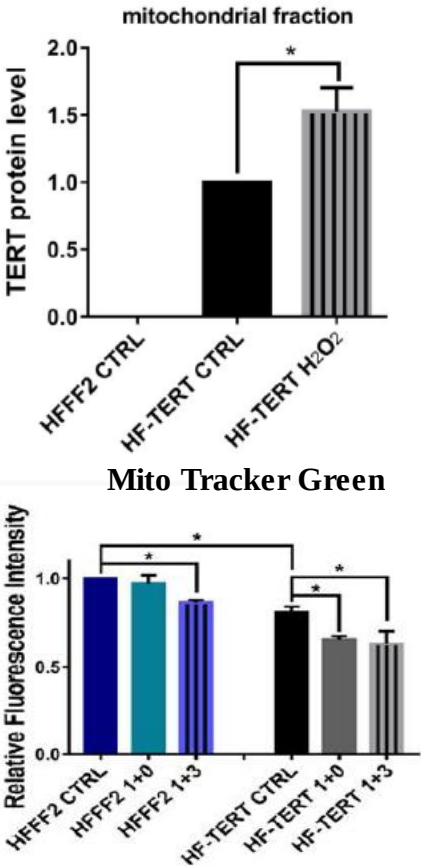
TERT Extra-Telomeric Roles: Antioxidant Activity and Mitochondrial Protection

Jessica Marinaccio ¹, Emanuela Micheli ^{1,*}, Ion Udriou ¹, Michela Di Nottia ², Rosalba Carrozzo ², Nicolò Baranzini ³, Annalisa Grimaldi ³, Stefano Leone ⁴, Sandra Moreno ^{1,5}, Maurizio Muzzi ^{1,5} and

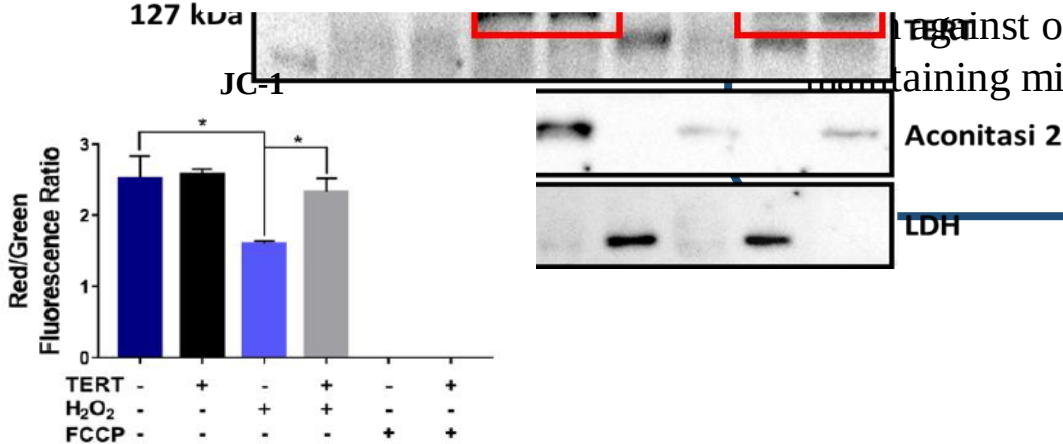
Mitochondrial Telomerase Reverse Transcriptase Binds to and Protects Mitochondrial DNA and Function From Damage

nal primary fibroblasts (HFFF2) which are cells without TERT

Judith Haendeler, Stefan Dröse, Nicole Büchner, Sascha Jakob, Joachim Altschmied, Christine Goy, M. Zeiher, Ulrich Brandt, Stefanie Dimmeler



ts—Here, we demonstrate that TERT is localized in the matrix of the mitochondria. TERT binds to NA at the coding regions for ND1 and ND2. Binding of TERT to mitochondrial DNA protects against e-induced damage.



against oxidative stress and its role in maintaining mitochondrial functionality.

AIM OF THE PROJECT

Therefore, the aim of my PhD project is to clarify the mitochondrial role of TERT, using U2OS and U2OS-TERT cell lines, through the following studies:

1.

STRUCTURAL INTERACTIONS

The direct binding of TERT to mtDNA sequences or the indirect interaction through mitochondrial DNA binding proteins.

2.

FUNCTIONAL ANALYSIS

The influence of TERT on mitochondrial DNA replication and trascription in basal conditions and following mitochondrial damage.

3.

FUNCTIONAL ANALYSIS

The possible TERT influence on mitophagy.

First year

EXPERIMENTAL PLAN

STRUCTURAL INTERACTIONS

I will use U2OS and U2OS-TERT-HA cell lines to confirm the binding of TERT to mitochondrial DNA.

IMMUNOPRECIPITATION

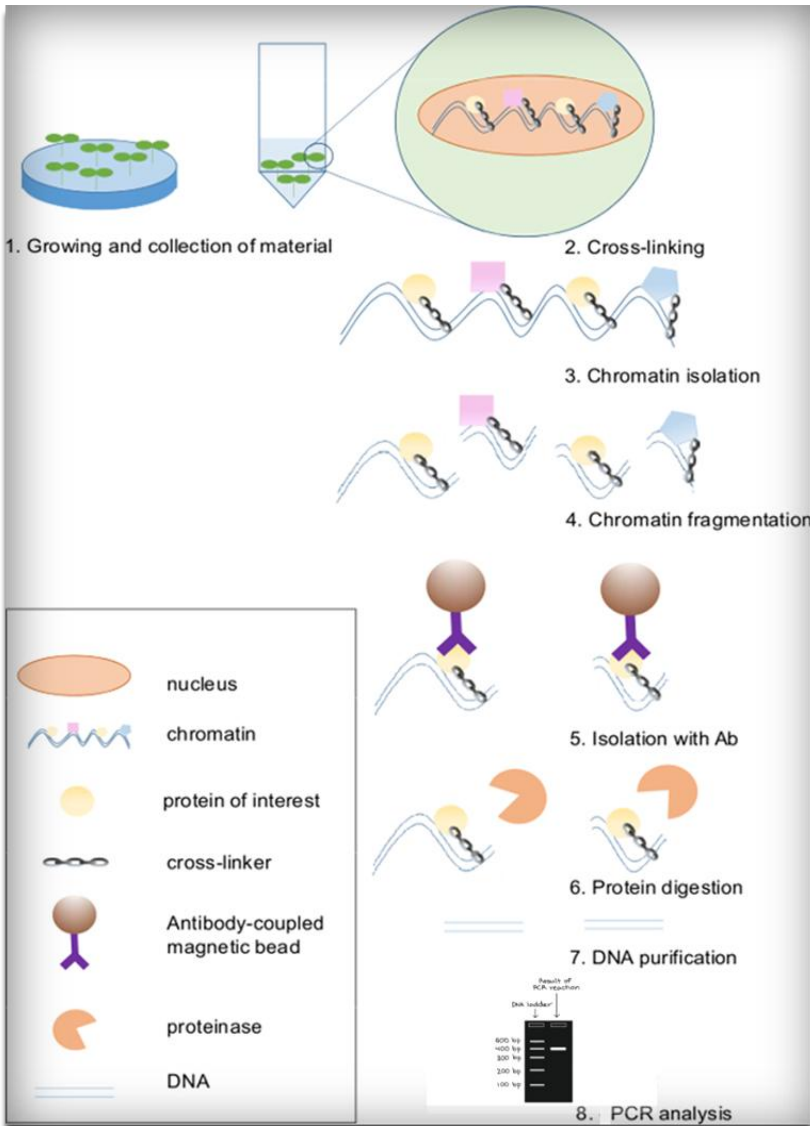
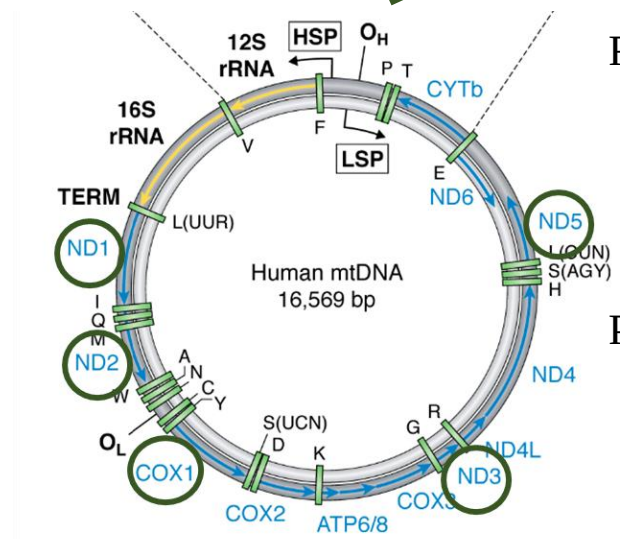
with anti-HA antibody

against putative protein interactors

RNA isolation

PCR on cDNA

To investigate TERT possible connection with mitochondrial DNA binding proteins such as TFAM or POLG





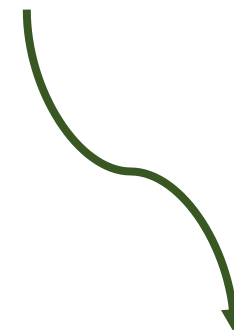
UNIVERSITÀ DEGLI STUDI DI SALERNO

EXPERIMENTAL PLAN

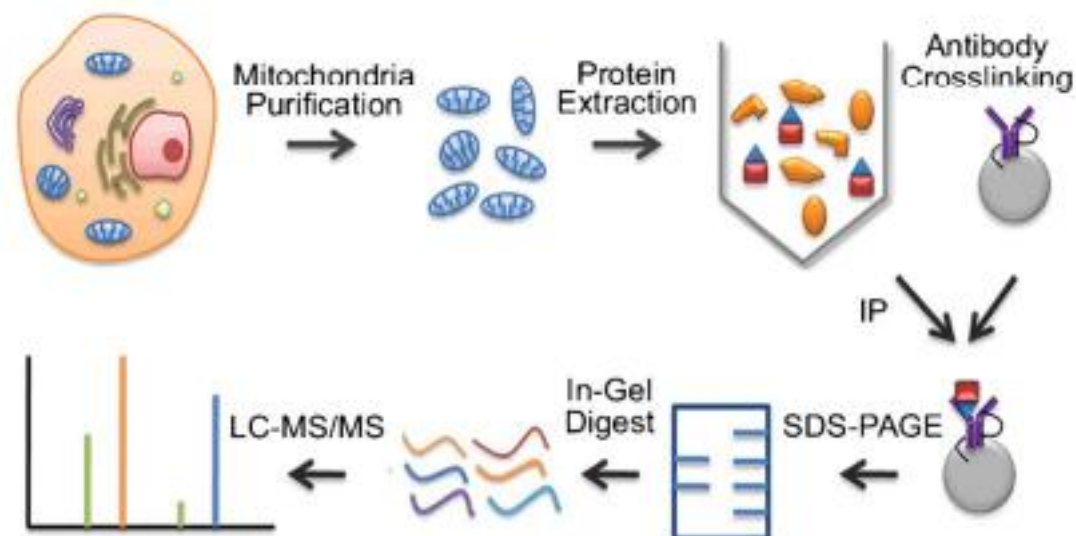
STRUCTURAL INTERACTIONS

First/Second
year

IMMUNOPRECIPITATION of the mitochondrial fraction



Mass spectrometry



Second
year

EXPERIMENTAL PLAN

FUNCTIONAL ANALYSIS

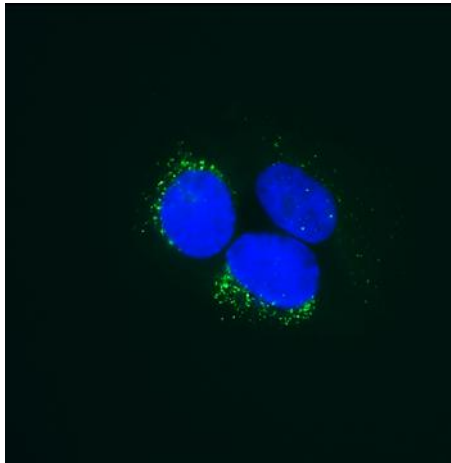
I will use U2OS and U2OS-TERT-HA cell lines to investigate whether TERT is involved in mitochondrial DNA replication

Aphidicolin

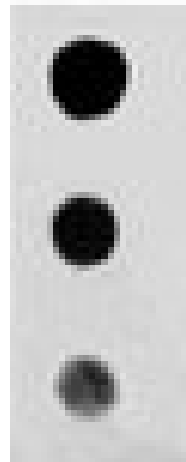
Bromodeoxyuridine

Inhibitor of
nuclear
polymerase

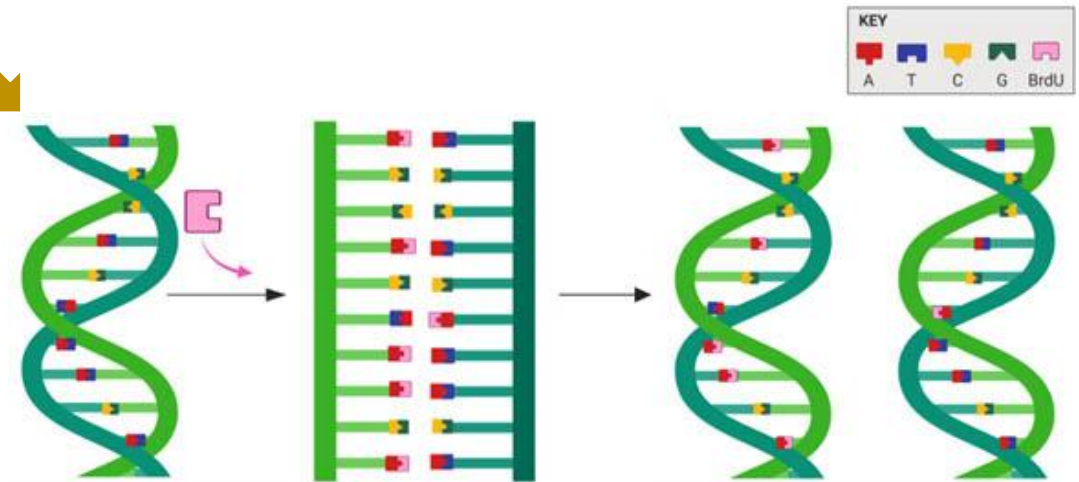
Immunofluorescence with
anti-BrdU antibody



Dot Blot



BROMODEOXYURIDINE



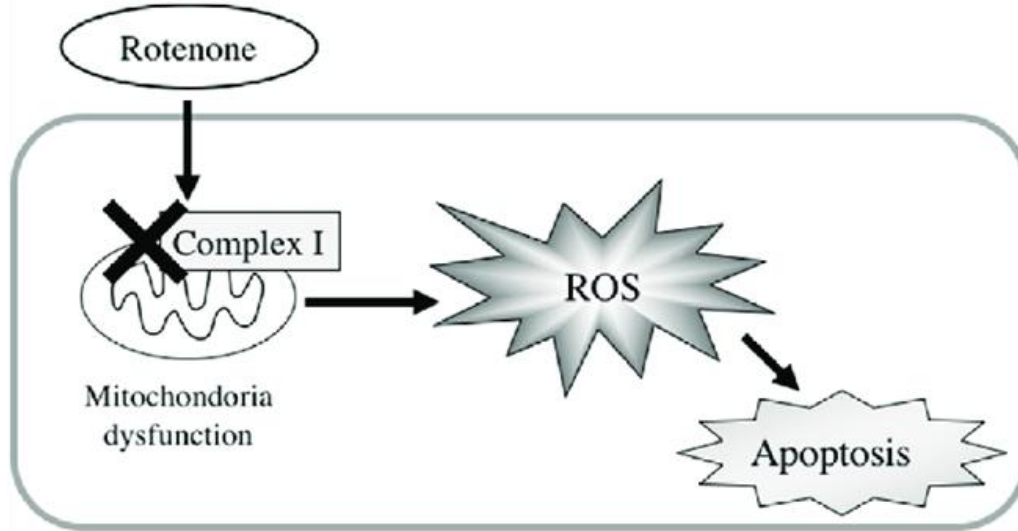
I WILL MAKE SURE THAT BrdU IS INCORPORATED IN mtDNA THUS
ESTABLISHING IF TERT CHANGES THE REPLICATION OF
MITOCHONDRIAL DNA.

Second year

EXPERIMENTAL PLAN

FUNCTIONAL ANALYSIS

I will induce mitochondrial damage utilising Rotenone, which is an inhibitor of electron transport chain complex I, causing ROS increase and mtDNA damage.



I will evaluate if TERT protein interferes with rotenone-induced damage by measuring mtDNA copy number.

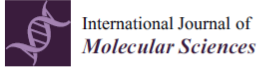
I will perform BrdU and Aphidicolin treatment to study if TERT is involved in the control of mtDNA replication

EXPERIMENTAL PLAN

FUNCTIONAL ANALYSIS

Third year

Considering . . .



Article

TERT Extra-Telomeric Roles: Antioxidant Activity and Mitochondrial Protection

Jessica Marinaccio ¹, Emanuela Micheli ^{1,*}, Ion Udroui ¹, Michela Di Nottia ², Rosalba Carrozzo ²,
Nicolò Baranzini ³, Annalisa Grimaldi ³, Stefano Leone ⁴, Sandra Moreno ^{1,5}, Maurizio Muzzi ^{1,5}
and Antonella Sgura ¹

Mitochondrial mass is lower in HF-TERT cells than in the control and decreases following hydrogen peroxide treatment.

In addition, the presence of TERT improves mitochondrial functions following treatment with hydrogen peroxide.

Therefore, it is possible that TERT could activate a mechanism to mediate the clearance of damaged mitochondria to maintain cellular functions.

To verify this hypothesis

I will assess if TERT could interfere with the mitophagy mechanism

EXPERIMENTAL PLAN

FUNCTIONAL ANALYSIS

Third year

MITOPHAGY

Mitochondrial mass

EGFP-mCherry fusion tag
on protein localised on
mitochondria

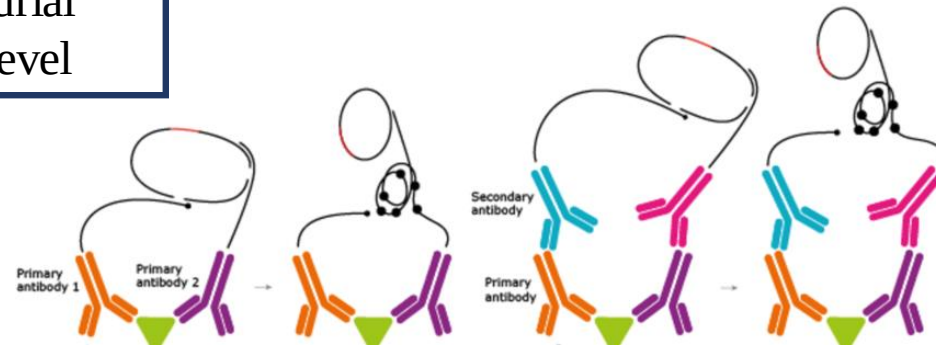
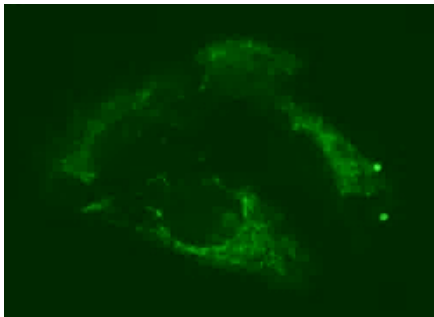
Mito Tracker Green

Possible changes in
mitochondrial
proteins level

Proximity Ligation
Assay

EGFP mCherry
Yellow fluorescence

EGFP mCherry
Red fluorescence



A large, glowing blue circle is centered on a dark blue background. The circle has a soft, ethereal glow, with the lightest part in the center and fading towards the edges. Inside the circle, the text "Thank you for your attention!!" is written in a dark blue, serif font, centered horizontally and vertically.

Thank you
for your
attention!!