eCOST – WG3 – Liver Task Group

Overview

- Leaders: Cervinkova Zuzana CZ / Sumbalova Zuzana AT
- Participants: Monsalve Maria ES, Palmeira Carlos PT
  - ➢ WE NEED TO GROW!

- INTERCOST – home grant
  - ➢ Possibility to finance your eCOST research
  - we succeded **INTER-COST LTC17044**

„Liver mitochondria - focus on Age, Lifestyle, Environment“

WG3 - liver

Team member		Institute
Gnaiger Erich	+ 2 more people	Austria, Innsbruck
Thyfault John	+ 5 more people	USA, University of Kansas, MC
Monsalve María	+ 6 more people	Spain, Madrid, CSIC-UAM
Arnaud Mourier	+ 3 more people	France, Bordeaux, IBGC
Spinazzi Marco	+ >30 more people	Belgium, Leuven, VIB KU
Garcia-Roves Pablo	+ 4 more people	Spain, University of Barcelona
Quiles José L	+ 3 more people	Spain, University of Granada
Červinková Zuzana	+ 5 more people	Czech Republic, Hradec Králové, CU

Name of your institute	
Address	
The head of the lab	Contact email
Team member	Contact email

Main current interests	
Linked to liver mitochondria:	**Other tissues:**
NAFLD	
Oxidative stress	
Liver toxic injury	
Impact of exercise and high vs low arobic capacity	
Ischemia reperfusion injury	
Liver regeneration	
Hepatoprotection	
Fibrosis	
HCC	

Major methodology	
Parametr:	**Methods:**
Respiration	HRR Oroboros 2K
Oxidative stress	HRP-Amplex Red, Fluorimetry (DCFDA and others)
Mitochondrial membrane potential	Fluorimetry(Safranine, TMRM, thodamine 123)
Mitochondrial swelling	Turbidimetry
Calcium retention capacity	Fluorimetry (Calcium Green)
Morphology	Histology, electron microcopy, live imaging
Gene expression	RT-PCR
Enzymes, proteins	Spectrophotometry, Western blot
Relative-telomere lenght	RT-PCR

			Notes:	Pubmed reference:
What experimental model do you use in you lab?	7	Isolated mitochondria	*What method of isolation do you use?*	
	2	Intact hepatocytes	*What method of isolation do you use?*	
	1	Permeabilized hepatocytes	*How do you permeabilize your cells?*	
	6	Liver tissue homogenate		
	2	Cultivated hepatocytes	*What type of cultivation do you use?*	
	2	Liver cell lines	*What cell lines do you use?*	
		Other..		
What species and sex do you use to isolate mitochondria/cells from?	1	Human		
	4	Rat		
	7	Mouse		
To the notes please specify the name of the species you use and the sex (F/M)	1	Rabbit		
		Guinea pig		
		Dog		
		Pig		
		Horse		
		Fish		
	1	Yeast		
		Drosophila		
		other..		
		other..		
How do you normalize your respirometry data obtained from isolated mitochondria/tissue homogenate?	5	Per amount of total protein	*What method do you use to estimate total protein?*	
	1	Using Flux control ratio	*What respiratory state do you use as a reference state?*	
	1	Per amount of specific mitochondrial protein	*What protein do you use and how do you measure it?*	
		Per activity of specific mitochondrial protein	*What protein do you use and how do you measure its activity?*	
		Per amount of mtDNA		
	1	Other..		
How do you normalize your respirometry data obtained from intact/permeabilized cells?	2	Per number of cells		
	2	Per amount of total protein	*What method do you use to estimate total protein?*	
	1	Using Flux control ratio	*What respiratory state do you use as a reference state?*	
		Per amount of specific cellular protein	*What protein do you use and how do you measure it?*	
		Per activity of specific cellular protein	*What protein do you use and how do you measure its activity?*	
		Other..		

Deliverables

- Standard Operating Procedures (SOPs)
- Database – data repository
- Quality Control

SOPs: Liver tissue

1. **Sample preparation**
   – solution, process
2. **Respiratory media**
   – composition, temperature,
3. **Respiratory protocols**
   – chemicals, concentrations
4. **Data evaluation**
   – standardization, templates

Critical issues

- **Separate approach to different experimental models**
  - ***Isolated mitochondria*** – **„Gold standard“**
  - **Tissue homogenate** – easy to get, easy to use... Could it be a good substitute? Limitations?
  - **Primary hepatocytes** – Comparison to cell lines
    - *Permeabilized cells*
    - *Intact cells in suspension*
    - *Harvested cultured cells*
  - **Cell lines derived from hepatocytes** (cancer cell lines)
  - **Human primary hepatocytes** – ethical questions

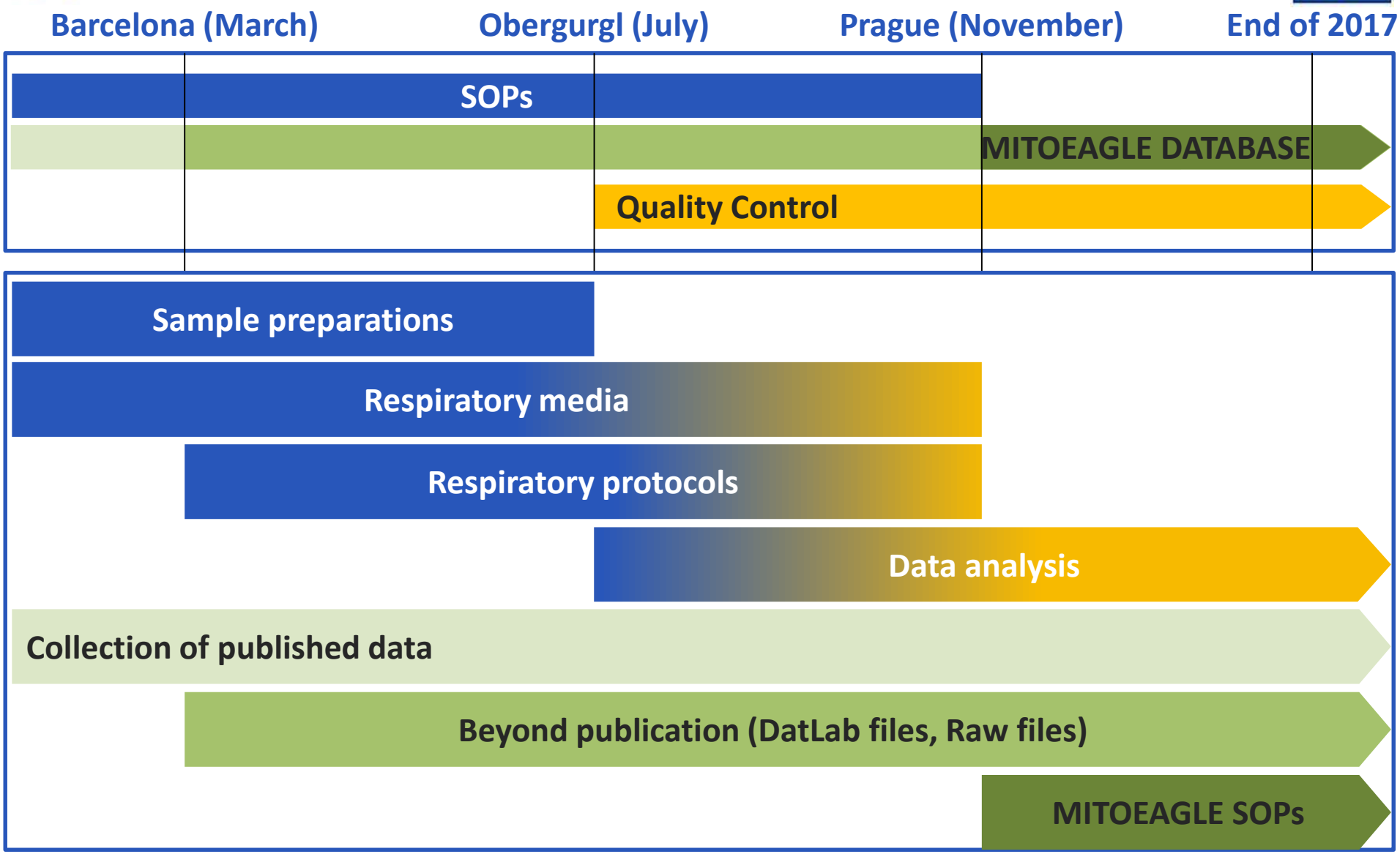
Necessary tools

- **Communication channel**
  - Crucial to maintain high efficiency of our work ?
- **Database**
  - Immediate data storage - ???
  - Data template
- **Networking itself (STSM,…)**
  - Important to train teams in sample preparations

    our experience – **University of Seville**

    Dr. Jordi Muntané - visited our lab ⇨ direct cooperation, common scientific paper

Working group 3 – Liver section



Delayed progress of work in comparison with our plan (WG3-liver)