Multiomics Approach on the Anhydrobiosis Pathway in Tardigrada (Water Bears) and its Implications for Space Travelling

Erasmus Education Programme
Origin, Evolution and Future of the Biosphere
Banyuls, 28th August 2014

Cécile Anne
Disa Backström
Antoni Beltran
Marcel Bläser
Shane Capper
Joseba García

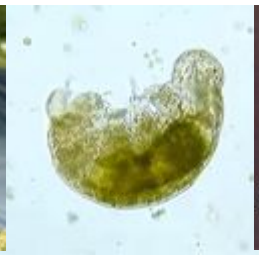
Content

- Introduction
  - Tardigrades
  - Anhydrobiosis
  - Pathways
- Proposed Experiments & Expected Results
  - Pathway screening
  - Multiomics
- Outlook
- Application

Tardigrades ("water bears")

Small 8 legged invertebrates
5+ million years
Incredibly resourceful
Many species & Habitats
Used in various areas of research

http://astronaut.com/if-earth-were-hosting-an-alien-species-this-is-what-it-would-look-like/



http://gifmansion.com/2115/tardigrade

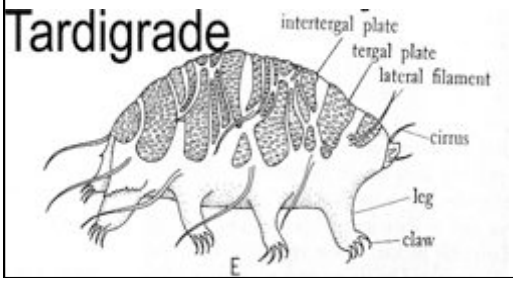
http://www.thrivenotes.com/wp-content/uploads/2012/08/tardigrade2.jpg

Why Tardigrades?

http://bioinf.nuim.ie/wp-content/uploads/2011/09/tardigrades_in_space.jpg

Ridiculously resilient

Nicole Ottawa & Oliver Meckes / Eye of Science



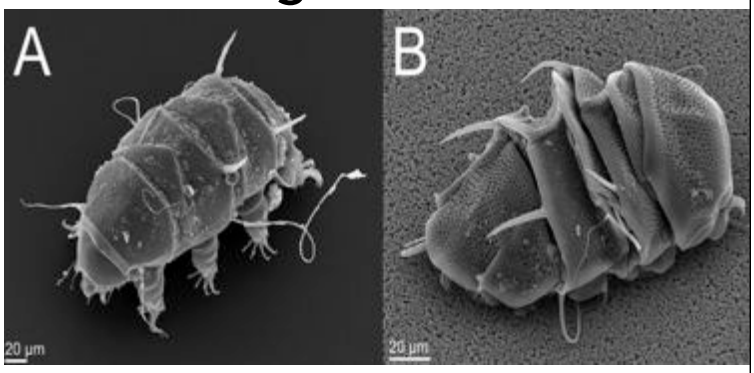
Extremophiles?

Prominent in previous research

Anhydrobiosis in Tardigrades

- A form of cryptobiosis induced by dehydration
- No signs of life activity
- Resume life after rehydration
- Resists:
  - Radiation
  - Alcohol, Methyl…
  - Dehydration
  - High/low temperatures…

Anhydrobiosis in Tardigrades



- Trehalose
- Antioxidants
- Aggregation
- Tun state
  - Cuticle contraction – Lipids
  - Body contraction
  - Invagination of limbs
  - Impermeability

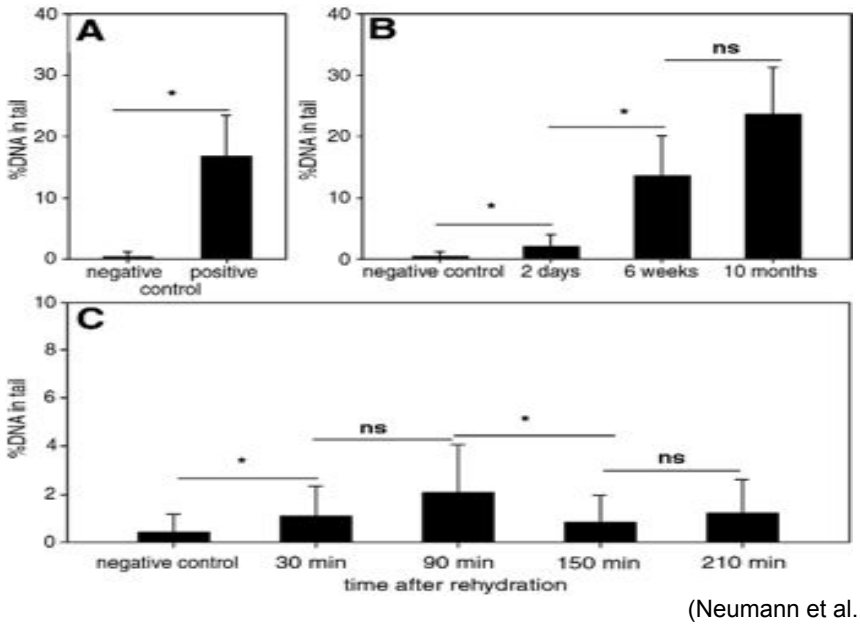
Anhydrobiosis in Tardigrades

- DNA damage
  - Reactive Oxygen Species
  - Irradiation
  - No DNA repair systems during anhydrobiosis
  - Nearly no DNA damage by dehydration

Anhydrobiosis in Tardigrades

Dehydrated time	Source	Survival/species
10 years	Sømme and Meier, 1995	
20 years	Jørgensen et al., 2007	
321 days	Rebecchi et al., 2006	38.8% Ramazzottius oberhaeuseri 74.9% Echiniscus spp.
72 h	Hengherr et al., 2008b	100% M. tardigradum
14 days	Schill et al. 2004	100% M. tardigradum
41 days	Jönsson et al., 2008	88.7% M. tardigradum
82.7 days	Hengherr et al., 2008a	133.2 days M. tardigradum
58 days	Suzuki, 2003	M. tardigradum

Anhydrobiosis in Tardigrades



(Neumann et al., 2009)

Anhydrobiosis in Tardigrades

DNA repair

- Nucleoside Excision Repair
  - Active state
- Base Excision Repair
  - Active state

Anhydrobiosis in Tardigrades

Stress proteins

- Chaperones "heat shock" proteins
  - During dehydration (Reuner et al., 2010a)
  - Non active (Reuner et al., 2010a)
  - Active → Non Active (Jönsson and Schill, 2007)
- Late Embryogenesis Abundant proteins
  - Active/non active states

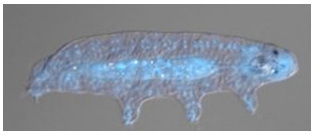
Too Many Cooks Spoil the Broth

Species	Source	Conditions	Result
Macrobiotus richtersi	Rebecchi et al. 2009	γ-radiation: 1.9 mGy + microgravity	All survive (dessicated)
Richterius coronifer	Jönsson et al. 2008	UV_{ABC} + space vacuum 10 days	1 survive UV_{AB} (desiccated)
Milnesium tardigradum	Jönsson et al. 2008	UV_{ABC} + space vacuum 10 days	68% survive UV_{AB} (desiccated)
Ramazzottius varieornatus	Horikawa et al. 2013	UV_C	Moderate tolerance (hydrated) High tolerance (desiccated)
Hypsibius dujardini	Horikawa et al. 2013	UV_C	Low tolerance (hydrated)

One space model Tardigrade should be chosen:
-High tolerance to space conditions
-Well studied

One Tardigrade to Rule them all

Species	Tolerance	Genome sequenced?	Survived space conditions
H. dujardini	Low	Yes	n.a.
R. varieornatus	High	Yes	n.a.
M. tardigradum	High	No, ~7000 ESTs	Yes



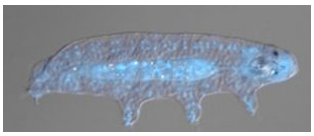
H. dujardini – tardigrades.bio.unc.edu/

M. tardigradum – nrm.se/

R. varieornatus – Horikawa et al. 2008

One Tardigrade to Rule them all

Species	Tolerance	Genome sequenced?	Survived space conditions
H. dujardini	Low	Yes	n.a.
R. varieornatus	High	Yes	n.a.
M. tardigradum	High	No, ~7000 ESTs	Yes



H. dujardini – tardigrades.bio.unc.edu/

M. tardigradum – nrm.se/

R. varieornatus – Horikawa et al. 2008

Goal of the Project

Tardigrades are able to survive extreme conditions, including space

The underlying pathway is not known – related to their ability to undergo anhydrobiosis

Our goal: To gain insight into the signalling pathway regulating anhydrobiosis in *M. tardigradum*, by using a multiomics approach

Image: bygonebureau.com/2011/03/23/fact-or-fiction-tardigrades/

Activation of the MAPK-Pathway in Nematodes in Response to Desiccation

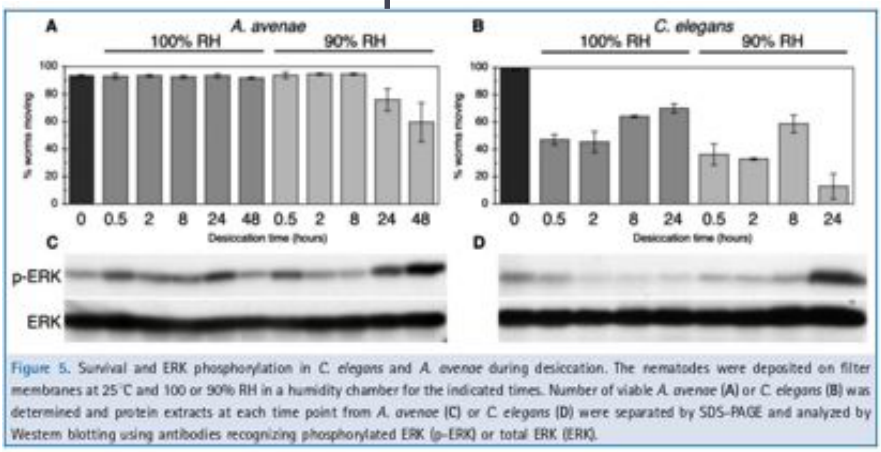
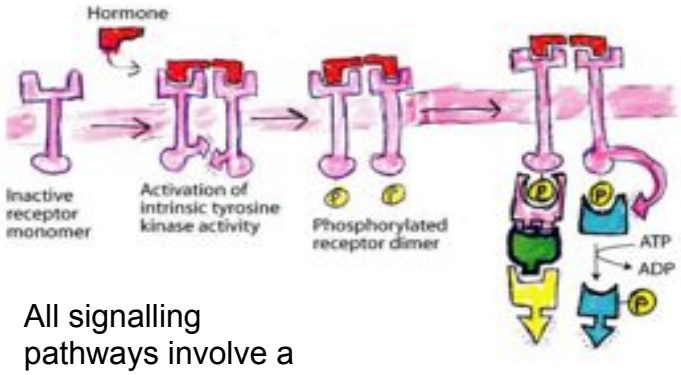


Figure 5. Survival and ERK phosphorylation in *C. elegans* and *A. avenae* during desiccation. The nematodes were deposited on filter membranes at 25°C and 100 or 90% RH in a humidity chamber for the indicated times. Number of viable *A. avenae* (A) or *C. elegans* (B) was determined and protein extracts at each time point from *A. avenae* (C) or *C. elegans* (D) were separated by SDS-PAGE and analyzed by Western blotting using antibodies recognizing phosphorylated ERK (p-ERK) or total ERK (ERK).

Perphaps the desiccation response in Tardigrades is also regulated by the MAPK pathway?

Huang et al. 2010

Signalling Pathways



All signalling pathways involve a signal, receptor, effectors and a downstream response

Downstream responses
Fast: Altered activity of cytoplasmic proteins
Slow: Regulation of gene expression → Altered activity of cytoplasmic proteins

Image: Johan Lennartsson, Uppsala University

MAPK-Pathway

The MAPK pathway is involved in many cellular responses and regulates for example growth, survival and proliferation of cells

By adding MAPK inhibitors the downstream response will be inhibited and the effect of the pathway on a phenotype can be studied

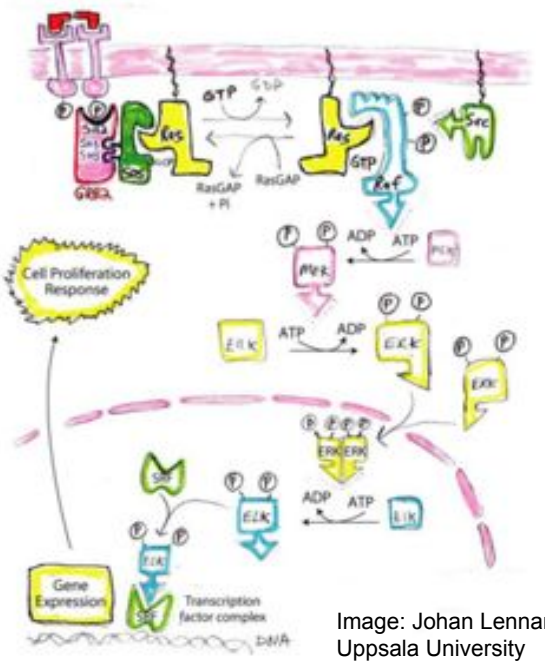


Image: Johan Lennartsson, Uppsala University

Proposed Experiments & Expected Results

What do we search for ?

Questions :
Which are the signalling pathways responsible for anhydrobiosis in Tardigrades?

Hypothesis :
The anhydrobiosis entry in Tardigrades is a regulated process that must be triggered by signaling pathways. MAPK has been shown to have a role in this process in other organisms.

What do we search for ?

To test our hypothesis :
We do a phenotypic screen by using kinase inhibitors to identify a potential pathway

We use a multiomics approach to confirm its role

- Transcriptomic
- Proteomics
- Metabolomics

Tardigrades culture

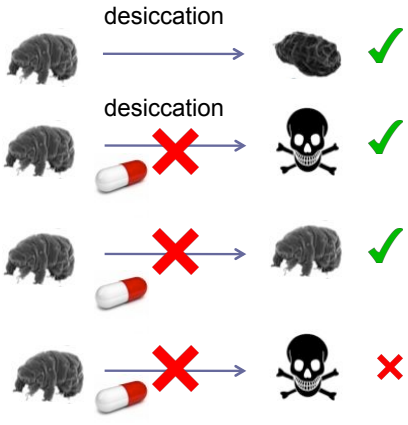
- Petri dish filled with a small layer of agarose and covered with spring water
- T° = 20°C
- Light/dark cycle of 12h
- Green algae and bacteria to feed them

Pathway screening

Variations of parameters	*Milnesium tardigradum*			
Treatment with pathway inhibitors	Y		N	
Dessiccation	Y	N	Y	N
Phenotype	Anhydrobiosis?			

Pathway Screening for Anhydrobiosis

We propose using **protein kinase inhibitors** to identify the responsible pathway or pathways for the entry into the anhydrobiotic state.



Normal entry into anhydrobiosis

The inhibitor prevents the entry into the anhydrobiotic state

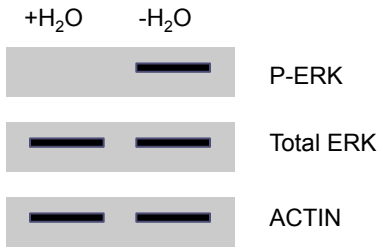
Control for the toxicity effects of the inhibitor (no dessication)

Pathway Screening for Anhydrobiosis

1. Inhibitor assay

Pathways to test	Inhibitor
MAPK	UO126
MAPK	PD98059
MAPK	SB203580
NF-κB	PDTC
PI3K-AKT	LY294002
PKC	SC3007, tamoxiphen

2. Confirmation of the pathway implication in desiccation conditions



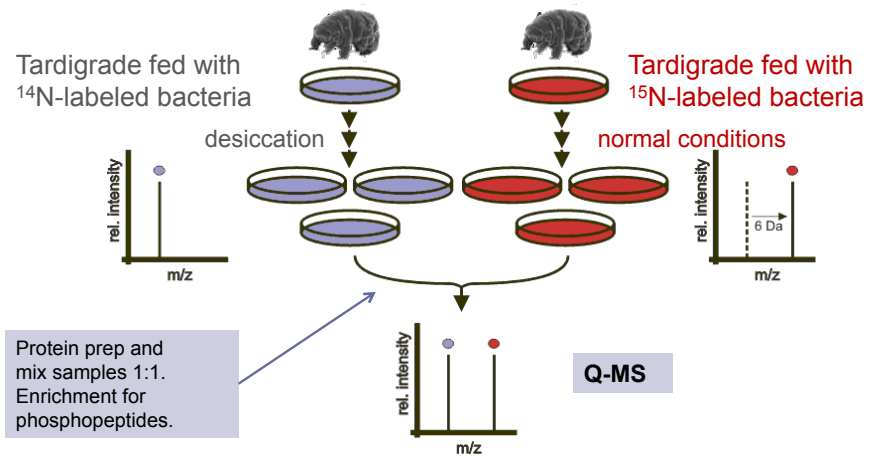
Multiomics

Multiomics

- 1. Phosphoproteomics → identification of molecules involved in the signalling network
- 2. Metabolome, Transcriptome and Proteome profiling → analysis of the response of the organism

1. Phosphoproteomics

- Enrichment for **phosphopeptides** to focus in the **signaling mechanism** rather than in the cellular response.

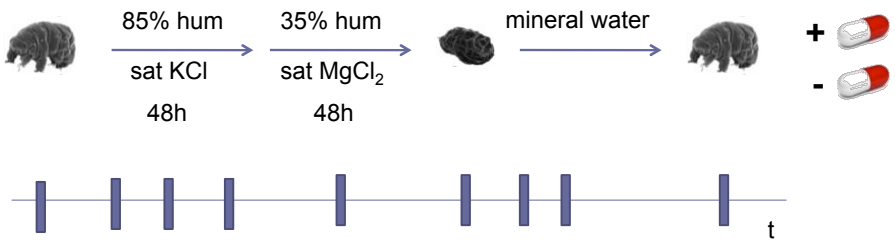


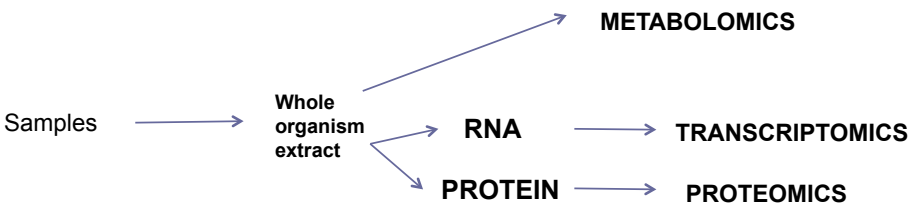
2. Metabolome, Transcriptome and Proteome profiling

Variations of parameters	*Milnesium tardigradum*							
Desiccation	Y				N			
Treatment with pathway inhibitors	Y		N		Y		N	
Samples at different time points	P	R	P	R	P	R	P	R

P : protein extraction R : RNA extraction

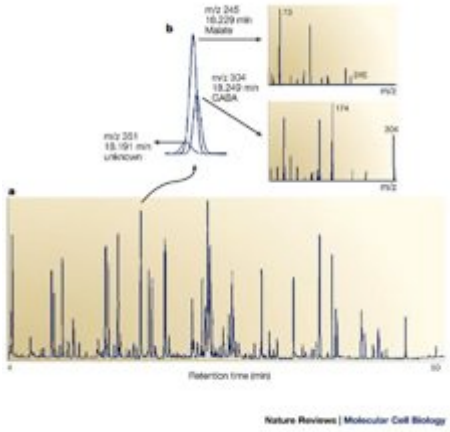
Sampling





Metabolomics

- Metabolic analysis is performed using GC-MS based metabolite profiling
- Network analysis and identification of subnetworks essential for anhydrobiosis



Metabolite profiling: from diagnostics to systems biology
Alisdair R. Fernie, Richard N. Trethewey, Arno J. Krotzky & Lothar Willmitzer. Nature Reviews Molecular Cell Biology 5, 763-769 (September 2004)

Transcriptomics

Differentially expressed genes

vs

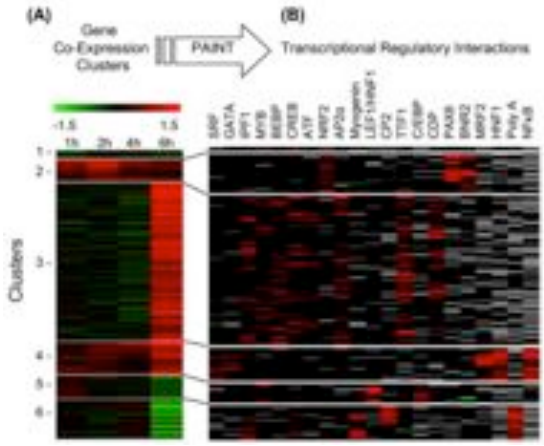
Transcriptome kinetics

Transcriptomics

- RNA-seq (Illumina) at the different time points
- Transcriptome kinetics in presence and absence of the pathway inhibitor

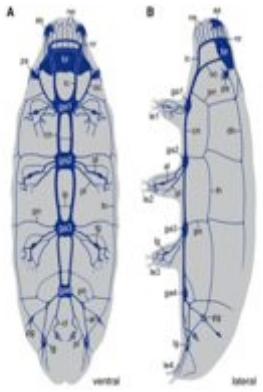
1. Identification of **gene expression programmes dependent on the pathway**.

2. **Clustering analysis** of the time series data to identify **co-regulated genes** in the anhydrobiotic transition.



Neuropeptidomics

- Analytic tool to identify peptides and proteins via mass spectrometry
- Dissect organs/ single cells



Mayer, *et al*. 2013

Expected Results

- List of bioactive and expressed peptides
- Animals in different states enable us to compare peptide-composition in hydrated- and tun-state
  - Peptides active in anhydriobysis pathway should be easily seen
- Control of MAPK pathway peptides

Tardigrade species 1				
name	sequence	$M[H]^{+1}$	Active	Dehydrated
Peptid A	GYRKPPFNGSIFa	1381.7313	X	X
Peptid B-1	RYLPT-OH	649.3778		X
Pptid B-2	EAPAQFQTESQMSQHLLTDEa	2289.0452	X	
Peptid C	SSNRSPSYRLRFa	1468.7768	X	X
Peptid D	ADPMMQGNQFSEHAAEAADNa	2133.8600		X
Peptid E	QTFQYSRGWTNa	1369.5908	X	X
Peptide F	DGGIRRGLIPFPRVa	1551.9367		X
Peptide G	SNPLQFDLRNEGPSFQSSASLF PFPRIa	3050.5483	X	

Outlook

- Sequence the full genome and transcriptome of *M. tardigradum*
  → Establish as model organism
- Identify the pathway responsible for anhydrobiosis
  - Identify all signalmolecules involved
  - Impact in Tardigrade physiology and UV-resistance with the help of all Omics
- Conserved in other species?

Application for space travel

- Modulation of related pathways to reinforce the resistance (e.g. UV) in other species (drugs)
- Implement DNA repair/ anhydrobiosis proteins in other animals (genetic engineering)
  - Farm animals for food supply during long space travels/ colonization
  - Even applicable in humans ?

Thanks for your attention!

References

Huang, Z., Banton, M.C., Tunnacliffe, A., 2010. Modeling Anhydrobiosis: Activation of the Mitogen-Activated Protein Kinase ERK by Dehydration in Both Human Cells and Nematodes. Journal of Experimental Zoology. 313A:660-670

Jönsson, K.I., 2007. Tardigrades as a potential model organism in space research. Astrobiology 7, 757-766.

Jönsson, K.I., Rabbow, E., Schill, R.O., Harms-Ringdahl, M., Rettberg, P., 2008. Tardigrades survive exposure to space in low Earth orbit. Current Biology 18. R729-R731.

Mali, B., Grohme, M.A., Förster, F., Dandekar, T., Schnoölzer, M., Reuter, D., Wełnicz, W., Schill, R.O., Frohme, M., 2010. Transcriptome survey of the anhydrobiotic tardigrade Milnesium tardigradum in comparison with Hypsibius dujardini and Richtersius coronifer. BioMed Central Genomics 11. 168

Rebecchi, L., Altiero, T., Guidetti, R., Cesari, M., Bertolani, R., Negroni, M., Rizzo, A.M., 2009. Tardigrade resistance to space effects: first results of experiments on the LIFE-TARSE mission on FOTON-M3 (September 2007). Journal of Zoological Systematics and Evolutionary Research. 49:98-103.

Welnicz, V., Grohme, M.A., Kaczmarek, L., Schill, R.O., Frohme, M., 2010. Anhydrobiosis in Tardigrades – The last decade. Journal of Insect Physiology. 57: 577-583.

References

Crowe, L.M., 2002. Lessons from nature: the role of sugars in anhydrobiosis. Comparative Biochemistry and Physiology A 13, 505–513.

Somme, L., Meier, T., 1995. Cold tolerance in Tardigrada from Dronning-Maud-Land, Antarctica. Polar Biology 15, 221–224.

Jorgensen, A., Mobjerg, N., Kristensen, R.M., 2007. A molecular study of the tardigrade Echiniscus testudo (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. Journal of Limnology 66, 77–83.

Rebecchi, L., Guidetti, R., Borsari, S., Altiero, T., Bertolani, R., 2006. Dynamics of longterm anhydrobiotic survival of lichen-dwelling Tardigrades. Hydrobiologia 558, 23–30.

Hengherr, S., Heyer, A.G., Ko¨ hler, H.-R., Schill, R.O., 2008b. Trehalose and anhydrobiosis in tardigrades—evidence for divergence in responses to dehydration. The Federation of European Biochemical Societies Journal 275, 281–288.

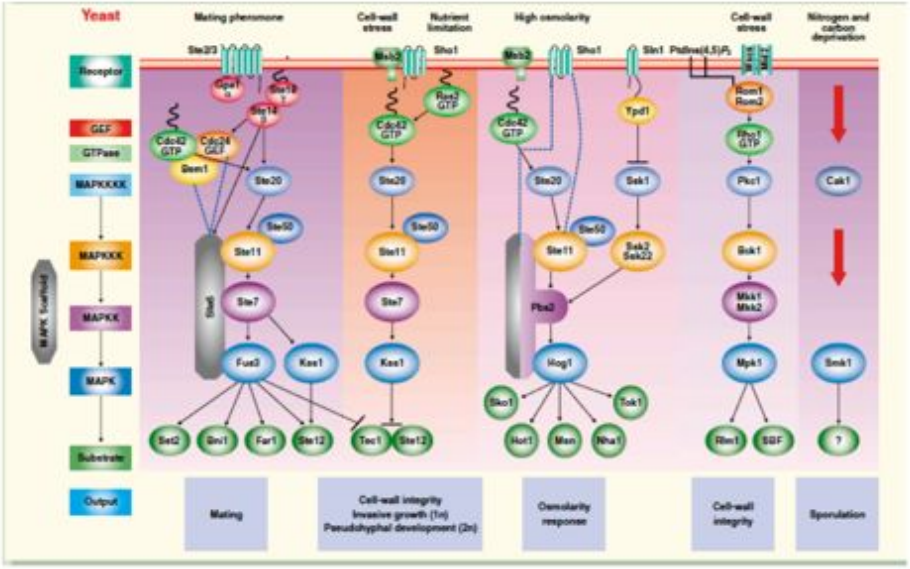
McGee, B., Schill, R.O., Tunnacliffe, A., 2004. Hydrophilic proteins in invertebrate anhydrobiosis. Integrative and Comparative Biology 44, 679–1679.

Hengherr, S., Bru¨mmer, F., Schill, R.O., 2008a. Anhydrobiosis in tardigrades and its effects on longevity traits. Journal of Zoology 275, 216–220.

Suzuki, A.C., 2003. Life history of Milnesium tardigradum Doyère (Tardigrada) under a rearing environment. Zool. Sci. (Tokyo) 20, 49–57.

Thanks for your attention!

Conservation of MAPK pathways from yeast to mammals



Conservation of MAPK pathways from yeast to mammals