



UNIVERSITÀ DI PARMA

3 GOOD HEALTH
AND WELL-BEING



SUSTAINABLE
DEVELOPMENT GOALS

2 ZERO
HUNGER



Higher plants response to metal-based nanoparticles: meta-analysis of data shows transcriptomics and proteomics rearrangements

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Sustainable
Nanotechnology
Organization

Research | Education | Responsibility

Metal-based nanomaterials



- Metal-based NMs can be applied in many different fields, because of their specific properties, chemical reactivity, optical features, conductivity
- But, they may enter the environment and potentially the food chains through plants
- In studying metal-based NMs it is relevant to distinguish those peculiar effects of nanoparticles, when they do not overlap with the effects caused by metal ions
- It is considered a purpose of risk assessment, to promote a safe and sustainable use of NMs

A new approach to make the technology more sustainable



- Existing data available on the effects of NMs in humans, in crop plants and in livestock are not sufficient to allow for a complete evaluation of their potential risks or of their safety
- For this it is necessary to go deeper into **functional toxicology** exploring the response at the level of specific genes or gene products
- and to find out **biomarkers** that can assist in risk assessment and in evaluation of toxicity, eventually connecting genes to proteins and to effects at the level of organisms
- Both are essential to enable a safe-by-design approach and in making this technology more sustainable and acceptable, especially when food production is concerned

But there is a source of new data which can help



Arabidopsis thaliana

Transcriptomics

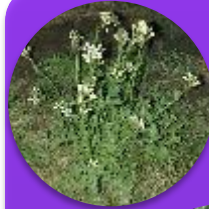
Ag NPs
TiO₂
CeO₂
ZnO
CuO
CdS QDs



Nicotiana tabacum

miRNA

Al₂O₃
TiO₂



Eruca vesicaria

Triticum aestivum

Proteomics

Ag NPs



Oryza sativa

Proteomics

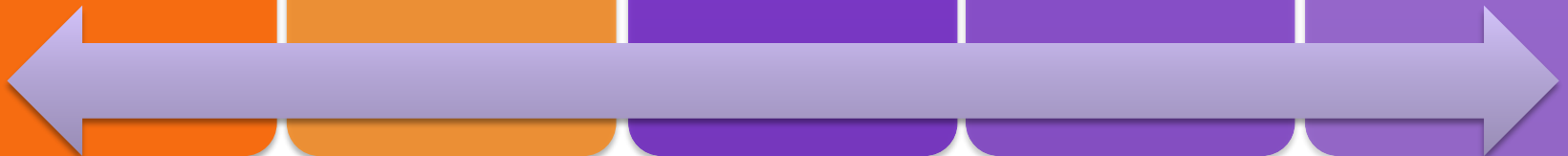
Ag NPs



Phaseolus vulgaris

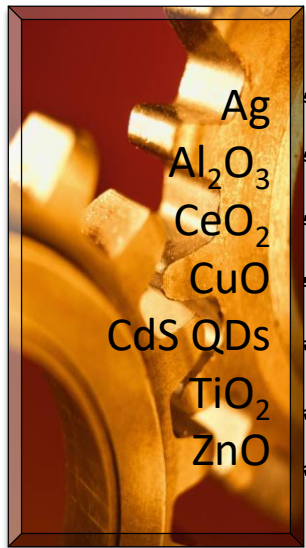
Proteomics

CeO₂

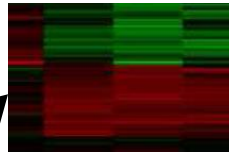


Our work in summary

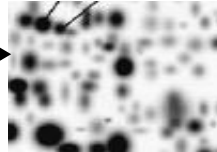
ENGINEERED
NANOMATERIALS



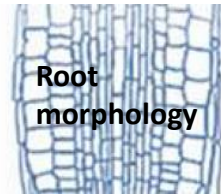
TRANSCRIPTOMICS



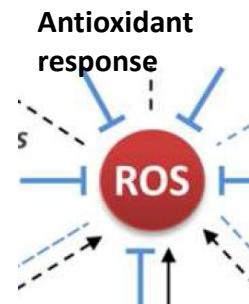
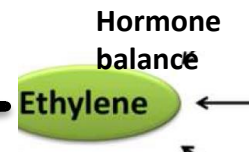
PROTEOMICS



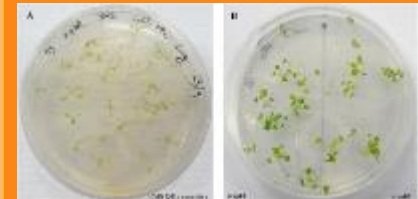
microRNA



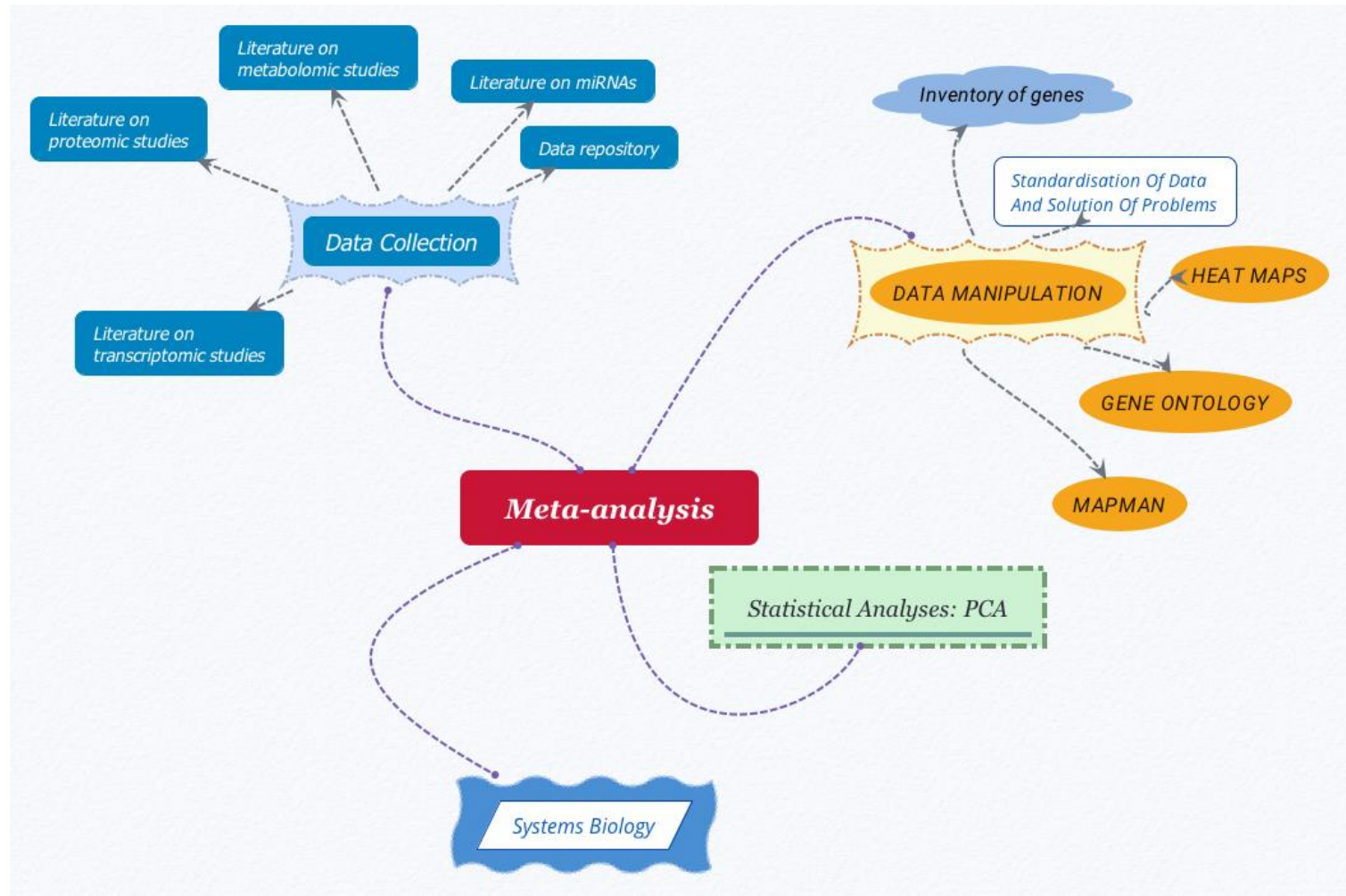
Root
morphology



VALIDATION OF DATA
THROUGH MUTANTS



The procedure used for meta-analysis



Problems with data heterogeneity

- Particle size
 - 5 – 150 nm
- Age of plants at treatment
 - 0 – 42 days
- Duration of the incubation
 - 2 hours – 102 days
- Concentration of nanoparticles
 - 0.2 - 500 mg/L
- Effects on plants
 - Negative (stress) or positive/neutral
- Tissues sampled
 - whole plants, roots, shoots, seeds
- Type of data reported
 - All data or just a subset

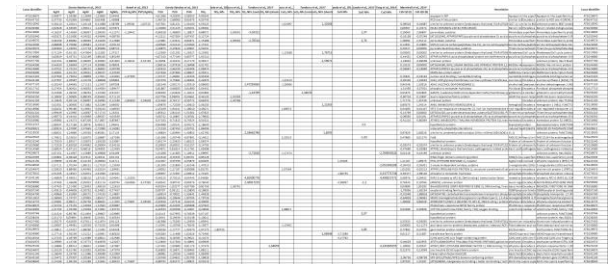


Repository of transcriptomics data

Data mining from the different papers and their supplementary material allowed the construction of a matrix comparing all genes found to be regulated in the different treatments

All data have been checked and the induction/repression level has been re-calculated, for homogeneity

The gene inventory contains information on over 25500 genes of *Arabidopsis thaliana* with data derived from 23 different experimental treatments. Hundreds of genes are differentially regulated



Gene ID	Gene Name	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9	Treatment 10	Treatment 11	Treatment 12	Treatment 13	Treatment 14	Treatment 15	Treatment 16	Treatment 17	Treatment 18	Treatment 19	Treatment 20	Treatment 21	Treatment 22	Treatment 23
AT1G01010	CHL1	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5
AT1G01020	CHL2	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5
AT1G01030	CHL3	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5
AT1G01040	CHL4	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5
AT1G01050	CHL5	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5

Linnaeus 4.0 – a new taxonomy

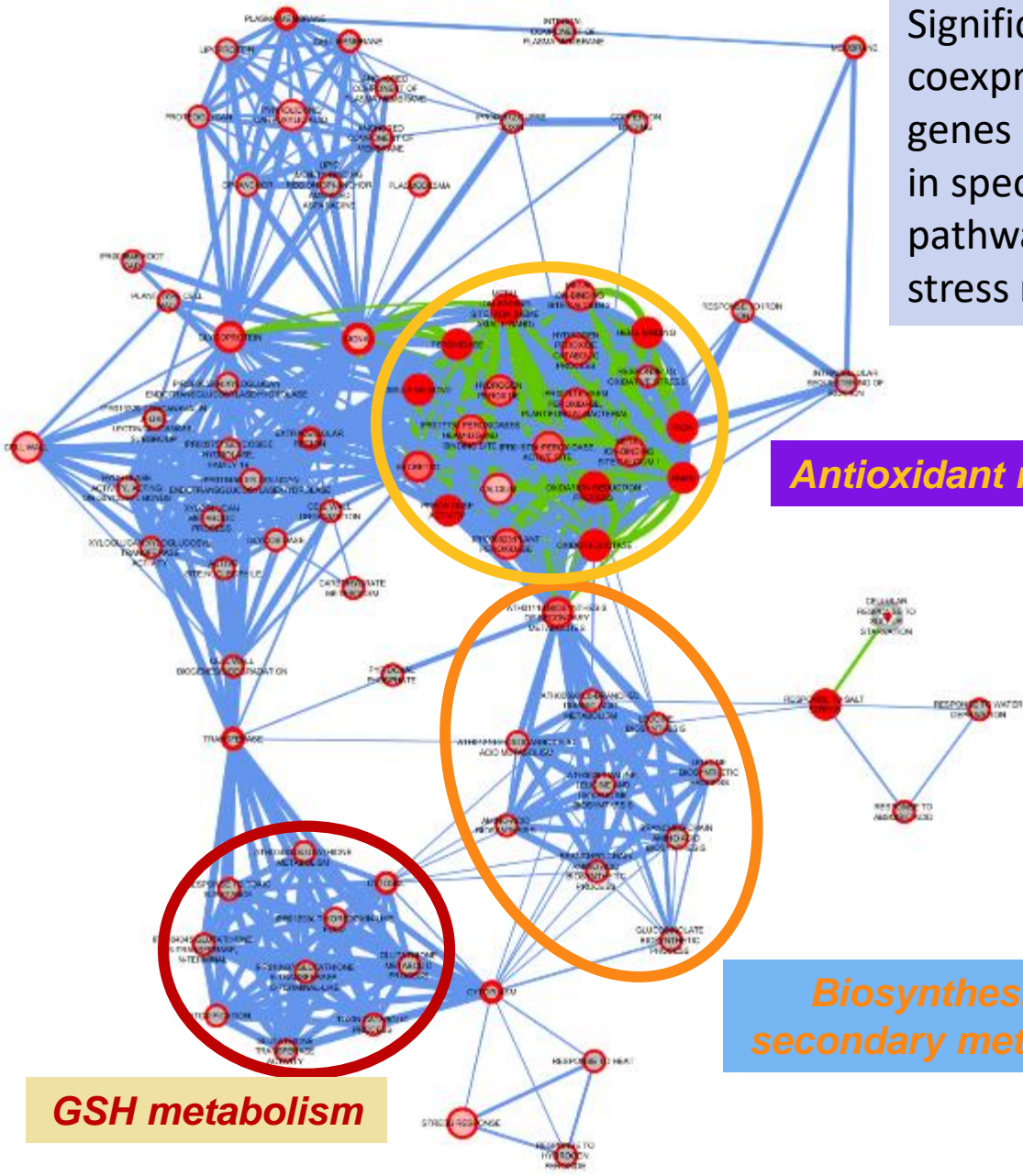
- Certainly «omics» provide thousands of data, a repository of incredible scientific value
- Is it the edge of the mechanistic approach, or rather a new kind of taxonomy, which only classifies changes, with less consideration for their physiological and biological role or effects?
- But without a link to the phenotype and to the behaviour of organisms, it is difficult to solve this problem

Genes and mutants

- The function of a gene can be extrapolated from the behaviour of the mutated alleles
- A population of Arabidopsis lines mutagenised with Ac/DS transposons from maize or with T-DNA insertions yielded just two mutants resisting to CdS quantum dots: *atnp01* and *atnp02*
- It is possible to combine the detail provided by hundreds of regulated genes with the observation that just few genes play a significant role in resistance to nanoparticles?
- Analysis of phenotype in mutants and a systems biology approach describing the interacting genes can provide the answer
- Network analysis and systems biology can identify genes/proteins which represent hubs for regulation
- Mutation in a hub gene can lead to a cascade of events, which become visible at phenotypic level

Common response in plants: Cds, QDs, CuO, ZnO

Significant coexpression of genes involved in specific pathways for stress response



Antioxidant response

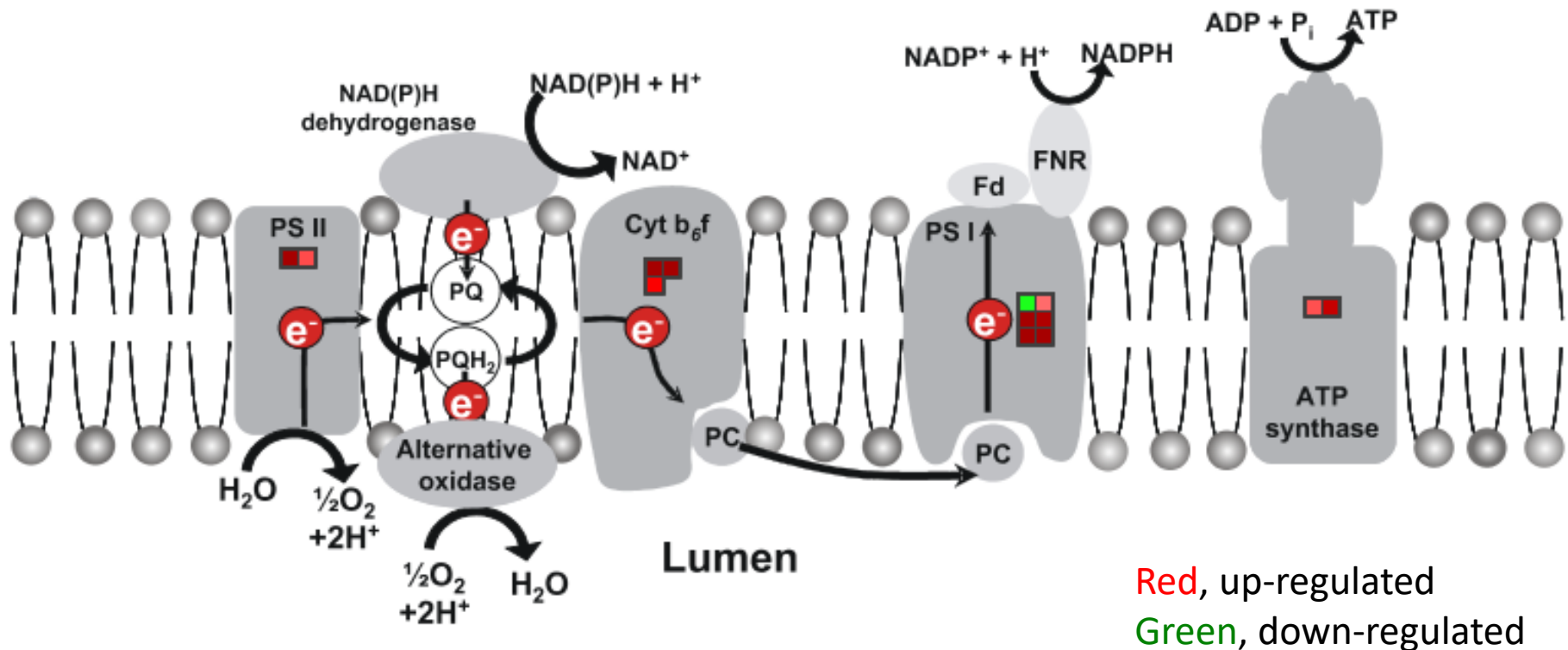
Biosynthesis of secondary metabolites

GSH metabolism

Specific response to CdS QDs: genes affecting photosynthesis

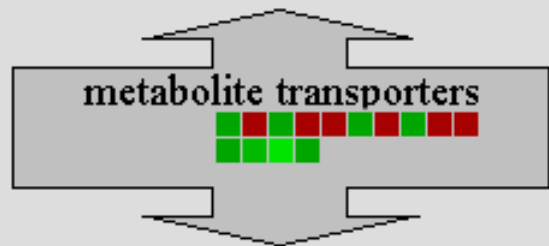
Genes involved in photosynthesis light reactions are up-regulated upon treatment with CdS Quantum Dots.

Treatment with Cd^{2+} ions does not affect the same genes.



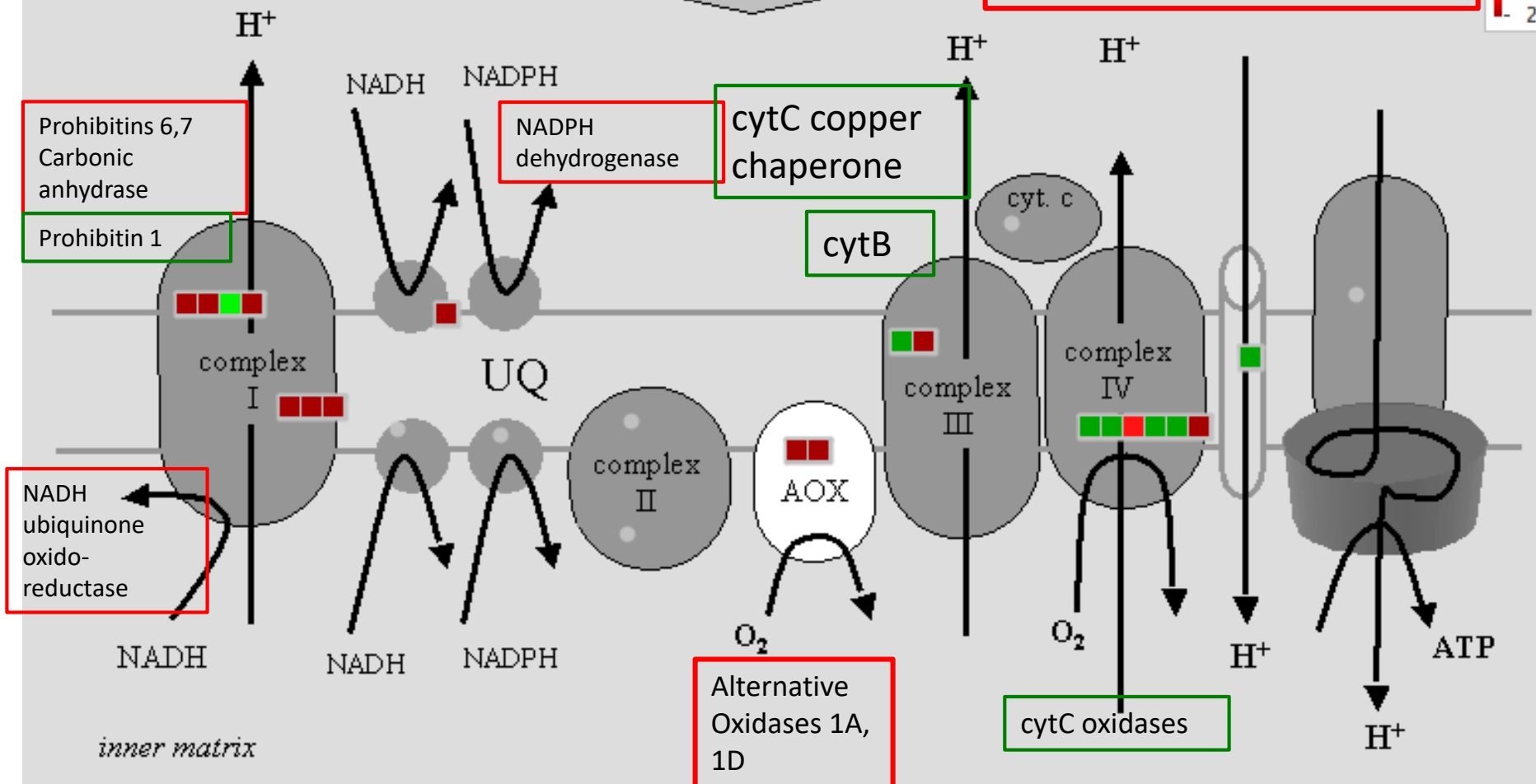
Red, up-regulated
Green, down-regulated

inter membrane space



Protein translocase TOM6 TOM7
Dicarboxylate carrier DIC3

ATP/ADP carrier
Dicarboxylate carrier DTC, DIT1



inner matrix

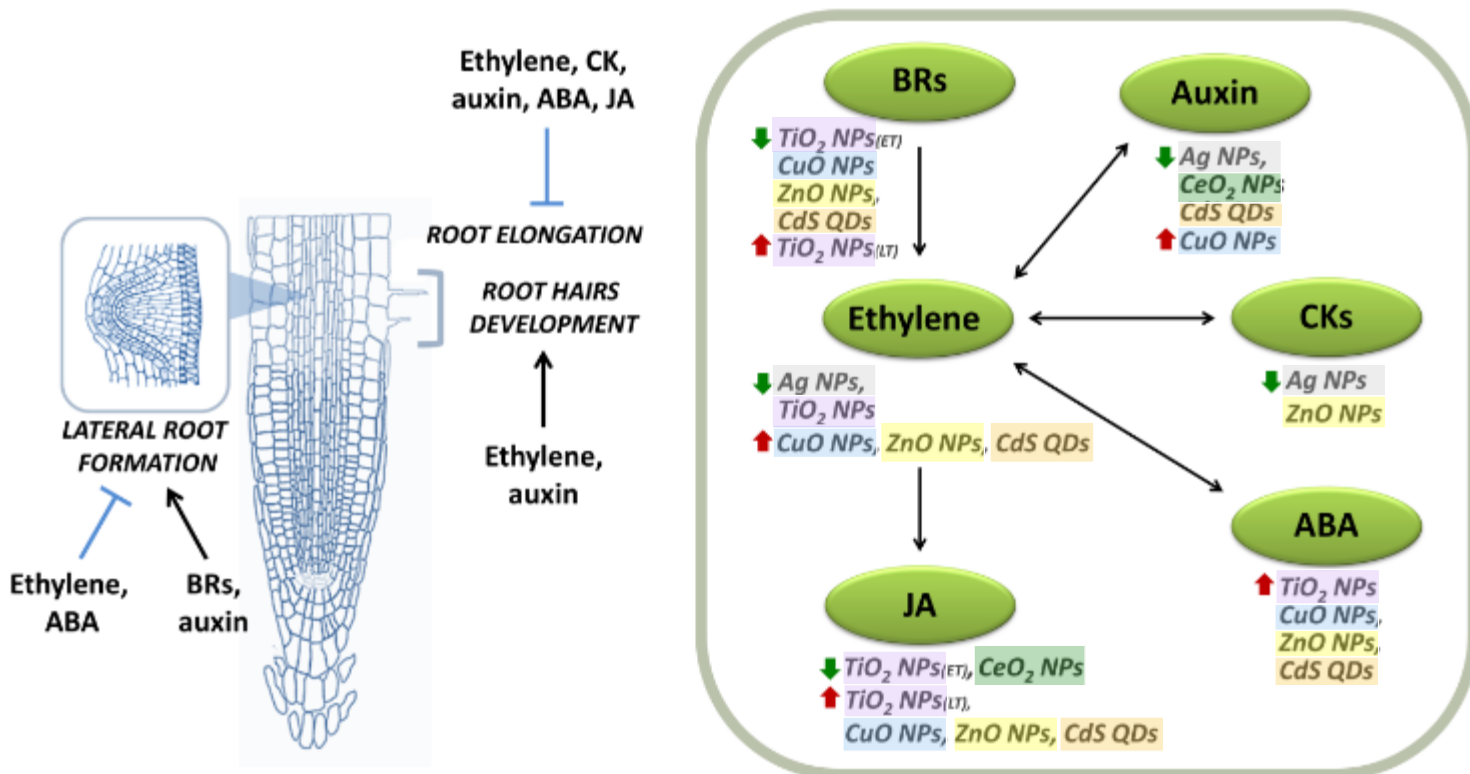
Representation of the main mitochondrial targets for nanoparticles effect [MapMan]

CytC Reductase complex

Uncoupling protein

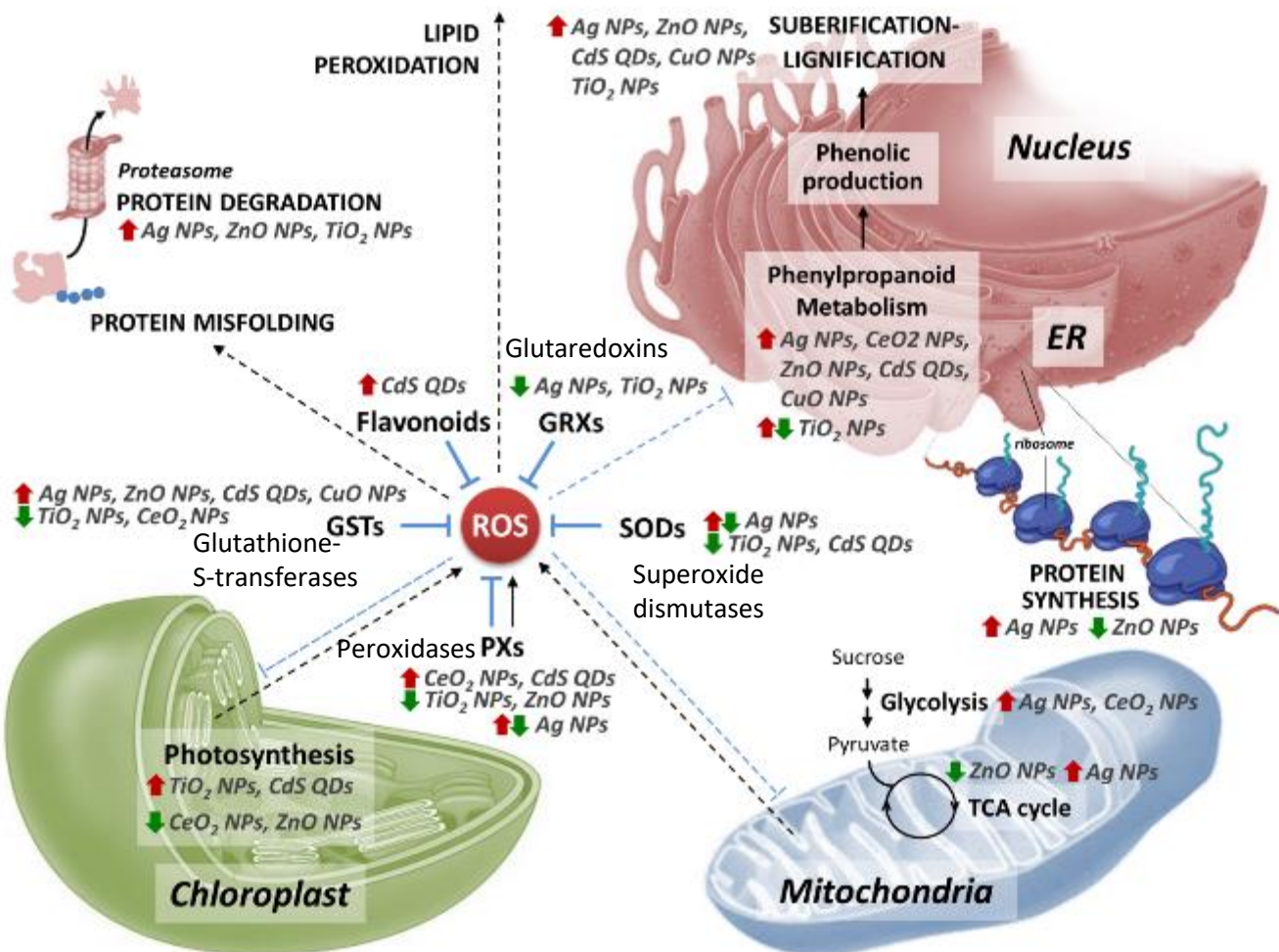
Common response in plants: hormones

Nanomaterials affect the expression of genes in the main hormone signaling pathways. The result is a modification of root development, inhibition of root elongation, inhibition of lateral root formation, appearance of root hairs.



BRs, brassinosteroids – CKs, cytokinins – ABA, abscisic acid – JA, jasmonic acid

Reactive Oxygen Species (ROS) are at the center of the stress response to nanomaterials. However, also organelles, defense proteins, lignification and protein degradation are involved. The existence of these mechanisms prompts additional considerations on the effect of nanomaterials on cells, tissues, organs. Increased knowledge will bring to a better use of nanomaterials, in terms of safety and sustainability.



What we have learned

Goals to be pursued

- Identifying biomarkers of exposure, genes or proteins which can be indicative of the stress condition induced by metal-based nanomaterials. One of these has been identified as a chloroplast localised function: electron carrier of the cytochrome b6f complex in the membrane
- Correlating transcriptomics, proteomics, metabolomics data to get a deeper insight into the molecular mechanisms underlying the response of plant cells, and to connect with the effects on phenotype
- Developing new strategies for risk assessment of nanomaterials, particularly in relation with the food supply chain: from the field to the fork
- Identifying also positive effects for exploitation in agriculture: nanopesticides, nanofertilisers, ...

THANKS!



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