Scientific report of the PhD project (third year)

Effects of metal-based nanomaterials on gametogenesis in different model organisms

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Nanotechnology is a rapidly growing field having potential applications in many areas. Metal-based nanoparticles (NPs), in particular, have received increasing interest due to their widespread medical, consumer, industrial, and military applications. However, as particle size decreases, some metal-based NPs are showing increased toxicity, even if the same material is relatively inert in its *bulk* form. One of the main concerns of these NPs is their potential genotoxicity, but the molecular mechanisms of action are still not well understood.

During the first part of my PhD in Biotechnology and Life Sciences, I studied the effect of a particular kind of NPs, Cadmium Sulfide Quantum Dots (CdS QDs), in the yeast *Saccharomyces cerevisiae*, focusing my research on the process of gametogenesis (called "sporulation" in yeast).

The presence of CdS QDs in the sporulation medium cause an alteration of the meiotic nuclear divisions in combination with a strong inhibition of spore morphogenesis, resulting in the formation of asci containing the meiotic products, but with the absence of refractile spores. The same effect was not seen using other types of metal-based NPs (ZnO, CuO, CeO_2) or a cadmium salt ($CdSO_4$). To determine whether the morphological alterations induced by QDs were accompanied by a transcriptional reprogramming of the gametogenesis process, a set of specific sporulation genes was analyzed by Real time PCR. In accordance with the phenotypic effects observed in response to CdS QD exposure, we found a down-regulation of regulatory genes expressed in the early-middle phase of the sporulation process and their downstream transcriptional targets.

During the second year of my PhD, I continued my work in yeast by identifying genetic alteration in the meiotic process monitoring the DNA content in yeast cells in different stages of the sporulation process. Cells were collected at different times, stained with propidium iodide (PI) and DNA content was measured by Fluorescence-activated cell sorting (FACS) by flow cytometry. Sporulated cells exposed to CdS QDs showed an altered DNA content profile, in line with the phenotypic observations obtained by fluorescence microscopy.

To better understand the molecular mechanisms involved in NP effect in sporulation process, we have studied and analyzed the composition of the “protein corona” formed on the surface of CdS QDs. Protein corona have an important role in biological activity and environmental fate of metal-based NPs. NPs are known to selectively adsorb proteins, to form a ‘corona’ bound tightly to their surface. Corona proteins which show a high affinity for the NP surface are exchanged slowly, and these so-called “hard” proteins form the innermost layer.

To investigate if the formation of hard corona proteins is linked to the effects seen on gametogenesis, proteins were extracted from cells during sporulation and incubated with CdS QDs with gentle agitation. Unbound proteins were removed by several washings, digested with trypsin and the samples subjected to liquid chromatography–mass spectrometry (LC-MS/MS) for protein identification. The most abundant protein found in the analysis are involved in the energetic metabolism and cell wall synthesis, the modulation of this proteins by the presence of CdS QDs suggests that energy metabolism is impaired by the ENP-induced stress, and could be a reason of the phenotypic observations.

The second part of this work, has been done in collaboration with the Connecticut Agricultural Experiment Station (CAES, New Haven, CT, USA). In this case my research was focused on another eukariotyc system, studying the physiological and molecular effects of different types of metal-based engineered nanomaterials (ENMs) on plant gametogenesis, with regards to the potential consequences on plant productivity of the crop *Cucurbita pepo* L. (zucchini). Literature is giving increased relevance to the beneficial effects that some ENMs may have on edible plants, when used as fertilizer or pest control agents. However, the lack of knowledge to the potential environmental effects and health risks are still limiting the widespread commerce and application of ENMs. Furthermore, the understanding of the ENMs effects related to the edible parts is still very limited. The ENMs taken into consideration in this project, together with their bulk counterparts, have potential application in agriculture as nanofertilizers or nanopesticides (CeO2, CuO). The study is performed in soil in order to bring to completion the entire life cycle of the plants. The ENM concentrations used in the study are chosen to maximize the response, without hampering the complete plant growth and flower development.

All of treatments at concentration tested showed no significant changes in the root or total plant biomass, as well as the morphological analysis of pollen performed by Environmental Scanning Electron Microscopy and pollen viability did not evidence any differences.

Inductively coupled plasma mass spectrometry (ICP-MS) analysis of metal content in flowers showed that Copper is able to translocate to the flowers, both in the bulk or in the nano form. Cerium content found in zucchini flowers is considerably lower than Copper, in agreement with past results showing a very low ability of Cerium to reach apical parts of the plant.

Likewise for yeast, a set of meiosis specific genes was selected and Real time PCR was performed from RNA extracted from anther undergoing meiosis. Two genes differentially expressed were found after CuO NPs treatment. In consideration of these results the main focus was placed on CuO NPs, performing a complete transcriptomic analysis of the different plant organs and tissues in zucchini.

Total RNA from roots, leaves and pollen of Zucchini treated with CuO NPS, CuO bulk and a salt form of copper was extracted and the samples were sent to IGA Technologies Services (Udine) for RNA-seq analysis. The preliminary results obtained from analisys of trancsriptomic expression in pollen showed a common response between the treatments performed, probably due to the physico-chemical properties of the materials. On the other hand, a side of the response can be considered more nano-specific and related to those mechanisms interested in oxidative stress prevention.