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Nucleo-mitochondrial interaction of yeast in response to cadmium sulfide quantum dot exposure

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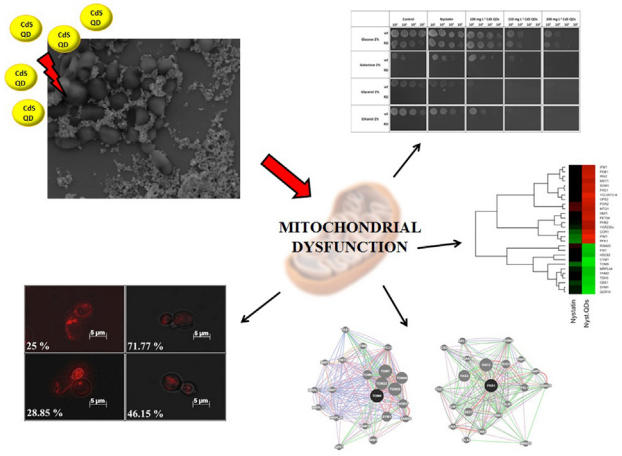
HIGHLIGHTS

- CdS QDs induce oxidative stress in yeast.
- CdS QDs disrupt mitochondrial membrane potentials and morphology.
- CdS QDs do not affect mtDNA content.
- CdS QDs modify the expression of genes involved in mitochondrial organization and function.
- Deletion of some of these genes induces either tolerant or sensitive phenotypes to CdS QDs.

GRAPHICAL ABSTRACT



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ABSTRACT

Cell sensitivity to quantum dots (QDs) has been attributed to a cascade triggered by oxidative stress leading to apoptosis. The role and function of mitochondria in animal cells are well understood but little information is available on the complex genetic networks that regulate nucleo-mitochondrial interaction. The effect of CdS QD exposure in yeast *Saccharomyces cerevisiae* was assessed under conditions of limited lethality (<10%), using cell physiological and morphological endpoints. Whole-genomic array analysis and the screening of a deletion mutant library were also carried out. The results showed that QDs: increased the level of reactive oxygen species (ROS) and decreased the level of reduced vs oxidized glutathione (GSH/GSSG); reduced oxygen consumption and the abundance of respiratory cytochromes; disrupted mitochondrial membrane potentials and affected mitochondrial morphology. Exposure affected the capacity of cells to grow on galactose, which requires nucleo-mitochondrial involvement. However, QDs exposure did not materially induce respiratory deficient (RD) mutants but only RD phenocopies. All of these cellular changes were correlated with several key nuclear genes, including *TOM5* and *FKS1*, involved

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in the maintenance of mitochondrial organization and function. The consequences of these cellular effects are discussed in terms of dysregulation of cell function in response to these "pathological mitochondria".

1. Introduction

Bakers' yeast (*Saccharomyces cerevisiae*) was the first widely adopted eukaryotic model, and near-complete descriptions of both its genome and proteome have been available for some time [1]. Its short life cycle (about 2 h at 28 °C), ease of culture both in liquid and on solid media [2], and availability of a large number of mutants [3] have together ensured the organism's wide-scale use in genetics and genotoxicology [4]. The development of knockout deletion mutants [5], in either haploid or diploid genetic states, has enabled pursuit of *in vivo* "genome-wide" toxicological approaches [6] which yields a thorough assessment of effects on nearly every gene [7].

Mitochondrial function is essential for *S. cerevisiae* growth on a medium containing oxidizable carbon [8]; for meiosis and sporulation [9–11]; for ascospore germination; for the ability to metabolize galactose [12]; and for apoptosis [13]. The majority of mitochondrial proteins are synthesized using nuclear DNA. There appear to be about 1000 proteins in yeast mitochondria [14], but only eight are encoded by mtDNA [15]. Since *S. cerevisiae* is capable of fermenting glucose without functioning mitochondria, respiration is dispensable. However, general mitochondrial function is not dispensable, because mtDNA mutants (*rho*$^{-}$ and *rho*^{0}) are developmentally compromised [16] and at least one mitochondrial pathway (iron-sulfur cluster assembly) is known to be essential for growth [17]. The non-essential nature of mitochondria in *S. cerevisiae* for glucose fermentation has been exploited to evaluate a number of mutations known to underlie specific human pathologies [18]. This approach is impossible using human or other animal cells that are unable to survive without full mitochondrial function.

In vitro studies of different types of QD on cells and tissues and *in vivo* whole organism studies have largely been conducted under conditions where toxicity was evident [19]. Only QDs with multiple shells (CdSe/ZnS QDs) have been used in longer-term experiments without significant negative effects on cell function. Smaller particles (such as CdS QDs) are generally more toxic, possibly due to an increase in Cd (II) ion release; however, in a previous paper [20] we showed that the toxic effects of CdS QDs did not require the release of Cd (II). QDs are known to act globally as disruptors of cellular redox homeostasis, having metalloestrogenic, nanogenomic and nanoepigenomic effects [19]. When administered to organisms under stress, QDs also trigger plasma membrane porosity and induce mitochondrial and nuclear damage [21]. In selected cells, QDs can induce autophagy [22] as a general stress response separate from apoptosis. This effect seems to involve supply of proteins from the mitochondrial membrane [23]. The results we have obtained for *S. cerevisiae* show the involvement of a nucleo-mitochondrial genetic network which accounts for most of the morpho-functional changes observed in response to CdS QDs exposure. Importantly, several of the genetic functions involved in yeast have orthologues in animal that can be used for similar studies on higher eukaryotes.

2. Experimental

"*Synthesis and characterization of CdS QDs*", "*Growth on different carbon sources*", "*Induction of petite cells and analysis of mitochondrial DNA markers*", "*Evaluation of ROS and glutathione levels*" and "*Cytofluorimetric analysis of yeast response to CdS QDs*" sections are reported in the Supplementary materials.

2.1. Yeast strains and CdS QD exposure

Most experiments used the bakers' yeast strain BY4742 (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0). Cells used for transcriptomic analysis were grown for 24 h at 30 °C in YPD liquid medium (1% w/v yeast extract, 2% w/v peptone, 2% w/v dextrose), supplemented with either 0.25 mg L^{-1} nystatin or 0.25 mg L^{-1} nystatin plus 75, 100 or 150 mg L^{-1} CdS QDs. A prior complete analysis of the CdS QDs minimal inhibitory concentration was carried out, using concentrations ranging from 0 to 250 mg L^{-1} (with and without nystatin); nystatin was added to encourage the uptake of the CdS QDs [20]. Other experiments utilized strain W303 (*MATα leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*), widely used to test the respiratory activity of mutants with compromised oxidative phosphorylation capability [24]. Culture purity was monitored by microscopic observation.

2.2. Staining with DAPI and Rhodamine B

For each treatment, cells were grown on liquid YPD for 24 h at 28 °C, after which an aliquot of ~3×10^7 cells was harvested by centrifugation (5000*g*, 5 min) and stained with DAPI (Sigma-Aldrich). The cells were visually inspected using an Axio Imager 2 microscope (Carl Zeiss, Oberkochen, Germany) equipped with a DAPI filter; excitation and emission wavelengths were 359 nm and 461 nm, respectively. The cells were also stained with Rhodamine B hexyl ester perchlorate (Life Technologies, Carlsbad, CA, USA), following Bornhövd et al.[25]; for microscopic observation, excitation and emission wavelengths were 528 and 551 nm, respectively.

2.3. Transformation with a mitochondrial directed mtRFP

The BY4742 strain was transformed with the plasmid pYX142-mtRFP [26] following Gietz and Woods [27]. This plasmid, provided by the laboratory of Prof. J. Winderickx, expresses a mitochondrial-localized red fluorescent protein (mtRFP) that allowed the analysis of the mitochondrial morphotypes produced under different treatment conditions. Cultures (10 mL) containing 10^4 cells mL^{-1} of the transformed strain were grown for 24 h in selective SC-LEU medium containing a non-repressive glucose concentration (0.6% instead of 2%), and in the presence of 100 mg L^{-1} CdS QDs or 20 μM $CdSO_4$. Fluorescence of mtRFP was detected at 588 nm with an RFP filter using an Axio Imager 2 microscope (Carl Zeiss). The percentages of different mitochondrial morphotypes were determined on approximately 500 counts per treatment.

2.4. Yeast deletion mutant collection analysis and transcriptomic analysis

The yeast deletion mutant collection analysis was performed as reported in Marmiroli et al. [20]. The transcriptomic analysis was performed on BY4742 cells grown for 24 h on liquid YPD without supplementation, with 0.25 mg L^{-1} nystatin, or with 0.25 mg L^{-1} nystatin plus 100 mg L^{-1} CdS QDS. To obtain the template required for the microarray-based transcriptomic analysis, total RNA was

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