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Selective Soft-X-rays interaction on the metabolism of eukaryotic cells

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Abstract

Novel techniques on yeast cells (laser-plasma generation of monochromatic soft-X-rays and computerized follow up by pressure transducers) give information on the effects of different doses, doses-rate, exposure times and X-ray wavelength on the metabolic activity of the cells, where a non-monotonic response and enzyme-driven oscillations are observed.

KEY WORDS: X-Rays, Yeast, Metabolism.

1. Introduction

Eukaryotic cells are extremely complex dynamical structures, therefore a reductionist approach has oriented modern research towards the study of simpler organisms (prokaryote cells), such as for instance *E. coli.* On the contrary we focus on yeast cells which are the smallest and simplest of eukaryotes with a genome three times the size of *E. coli*, but only two-hundredths the size of human genomes, and which behave much as human cells do.

The combination of compactness, complexity, similarity to other eukaryotic cells and the sheer experimental practicality make yeast ideal for several purposes; on one side yeast is the first eukaryote to have its genome mapped, on the other, yeast metabolic activity (e.g. glycolysis and fermentation producing CO_2) permits to investigate several fundamental biological mechanisms which are controlled by enzymatic activities (sometimes electromagnetically driven) and localized well outside the nucleus, into several specific compartments.

In the past, X-rays interaction with cells has been mainly limited to the study of the dose effect on their survival rate and genetic material response, aiming at a definition of a standard (possibly) monotonic feature. Only recently it has been realized that energetic X-rays will deliver energy into the cell in a sparse manner, introducing damages that cannot be easily identified in terms of the definition of the involved compartment and of the elementary bio-chemical-physical interactions.

2. Methods and results

We introduce the coupling of two novel techniques: (1) laser-plasma generation of monochromatic soft-X-rays (< 1.5 keV) [1], (2) automatic and computerized follow up of gas production from yeast (*Saccharomyces cerevisiae* Hansen) cell glucose-driven metabolic activity with high sensitivity [2]. They have made possible: (1) to selectively deliver the radiation dose in the relevant cell compartments, (2) to monitor the cell metabolic activity in a non-invasive manner, while tracking basic phenomena.

This matches also with the fact that the nucleus is not the only relevant cell compartment if we are not looking for genetic effects; actually the properties of membrane and cytoskeleton-cytoplasmatic structure involving the cell-cell communication and the cell metabolic activity, are as much as important too. X-rays – wall cell interaction leads to precious information on fundamental facts in the kingdom of the cell immune system.

The unique characteristics of the rigid and robust wall-membrane complex of yeast, has permitted us to add a valuable shielding effect on the mean free path of the impinging X-rays, making the task easier to identify the very cell structures which are more likely the targets of the irradiation procedure.

Experimental results give information on the effect of different doses, dose-rates, exposure times and X-ray wavelengths on cell metabolic activity. Data show non-monotonic response as a function of the dose that enlighten the complexity of "pathway separation". Details on the competing metabolic pathways emerge from the use of different nutrients and the analysis of different repair processes.

The proposed technique make it possible to extract quantitative results on the presence of metabolic oscillations that are linked in our investigation to the fundamental enzymatic activities at work in the cytoplasm [3]. The identification of oscillating periods allows a strict correlation between metabolic activities and past X-ray exposure. In fact the former involves the Pasteur effect which is the control exerted by respiration over glycolytic rate. When cells are growing fermentatively under anaerobic conditions, the rate of glucose utilisation will

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be high. A switch to aerobic conditions results in the onset of respiration with a consequent rapid decrease in the rate of fermentation (glycolysis) in order to control a balanced supply and storage of ATP. Despite the several metabolic pathways possible, canonical wisdom associates this effect to one enzyme only: the allosteric regulation of the key enzyme phosphofructokinase (PFK) which allosteric regulation is responsible of the oscillations observed [4]. Therefore an X-ray induced modification in their behaviour can be related to X-ray induced PFK behaviour, thus giving clues to an elementary interaction of general interest.

Finally the obtained information on the recovery mechanisms of these basic eukaryotic systems give some hint on possible selective action on the basic cellular elements of human immune system, with major attention devoted to surface based process. Actually yeast cell wall may mimic more closely the intercellular matrix of animal tissues, being composed of a structural glucan and mannoproteins which have their counterpart in blood group substances and immunoglobulins [5]

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