ESRF	**Experiment title:** **Chemical element mapping in yeast cell models of amyotrophic lateral sclerosis**	**Experiment number**: MD-56
Beamline: ID-21	**Date of experiment**: from: july 1st, 2004 to: july 6th, 2004	**Date of report**: 10/11/2004
Shifts: 15	**Local contact(s)**: Barbara Fayard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Ortega Richard, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France

*Devès Guillaume, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France

*Roudeau Stéphane, LCNAB, CNRS, Université de Bordeaux 1, Gradignan, France

Report: The aim of this experiment was to investigate the intracellar distribution of trace elements and particularly iron in yeast *Saccharomyces cerevisiae* models of amyotrophic lateral sclerosis (ALS), using synchrotron X-ray microprobe at ESRF beamline ID-21.

ALS is a a fatal human neurodegenerative disease of unknown mechanism but Cu,Zn superoxide dismutase (CuZnSOD) mutations have been implicated in subsets of ALS familial cases. *S. cerevisiae* strains expressing human CuZnSOD mutants were constructed to study the biochemal properties of CuZnSOD mutants and their cellular effects. It has been suggested that theses mutations could induce oxidative reactions leading to the release of iron from Fe-S clusters proteins and resulting in the propagation of oxidative damage catalysed by labile Fe.

In this experiment, yeast cells expressing a human CuZnSOD mutant, A4V, were compared to yeast cells expressing the human wild type CuZnSOD (hWT) to study the effect of A4V mutation on iron intracellular distribution. Iron is thought to be preferentially stored in yeast vacuoles.Yeast vacuoles were stained with a specific blue fluorescent dye. This allowed the precise localization of yeast vacuoles by fluorescence and light microscopy before synchrotron X-ray microprobe analysis.

Scanning X-ray fluorescence microprobe analysis was performed on cryofixed-lyophilized yeasts cells using a 7.19 keV energy beam with 0.4 x 0.7 μm^2 spatial resolution. Distribution maps of P, S, Cl, K, Ca and Fe were obtained for A4V and hWT cells and correlated to fluorescence microscopy pictures (Fig. 1). For A4V and hWT cells S, Cl and K distributions were homogenous and representative of the total cell surface. P and Ca were co-localized in the specific cell area corresponding to the vacuole, as indicated by comparison with vacuolar staining micrographies. Yeast vacuole is known to store P as polyphosphates, themselves able to bind Ca.

Fe cellular distribution was more complex. Fe was found in the whole cell volume but its distribution was not homogeneous. Quantitative results were calculated in µM assuming that yeast cells and vacuoles are spherical objects. In both cell types, Fe concentration was about two times higher in the vacuole than in the extra-vacuolar space which includes cytosol and nucleus (Fig. 2). In addition, Fe was found to be localized in small extra-vacuolar structures, and especially at high local concentration in A4V mutants (Fig. 3). These data are preliminary but clearly suggest an alteration ofin iron metabolism in A4V mutants of CuZnSOD expressed in yeast. This modification may be related to the destruction of Fe-S clusters proteins as a consequence of an increase in free radicals production. The exact nature of observed ‘extra-vacuolar iron-rich structures’ in A4V mutants remains to be explored.

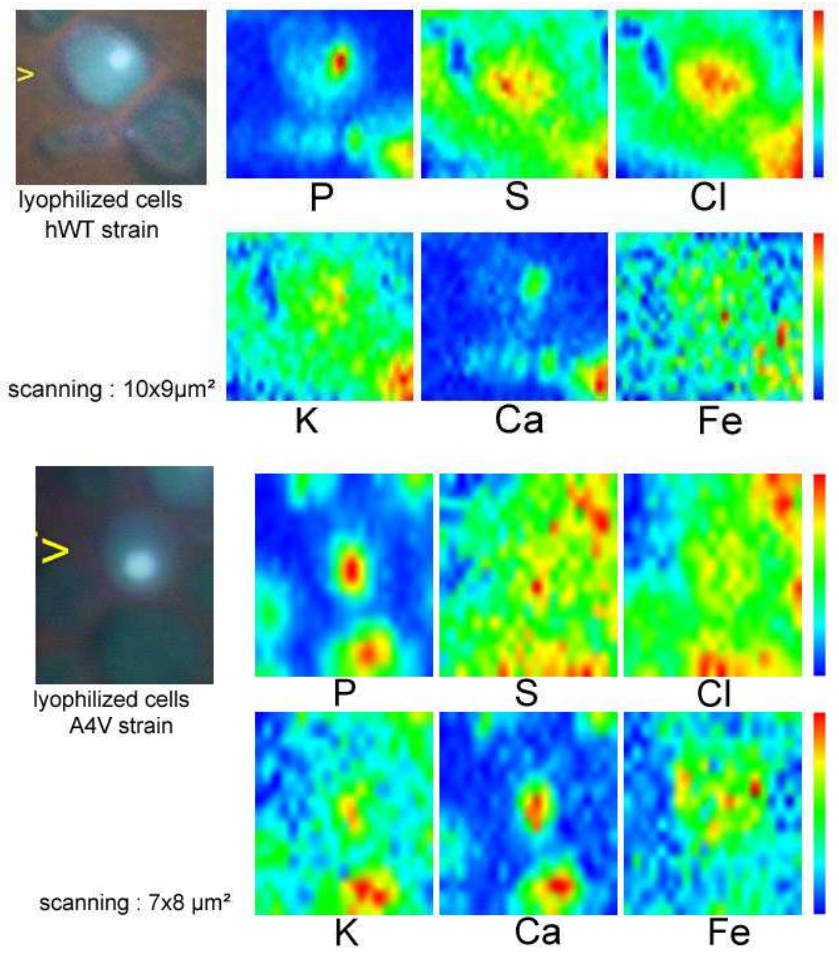

Figure 1. P, S, Cl, K, Ca and Fe intracellular distribution in hWT and A4V yeast cells. Micrography on the left show the corresponding position of vacuole (blue) observed by conventional fluorescence microscopy.

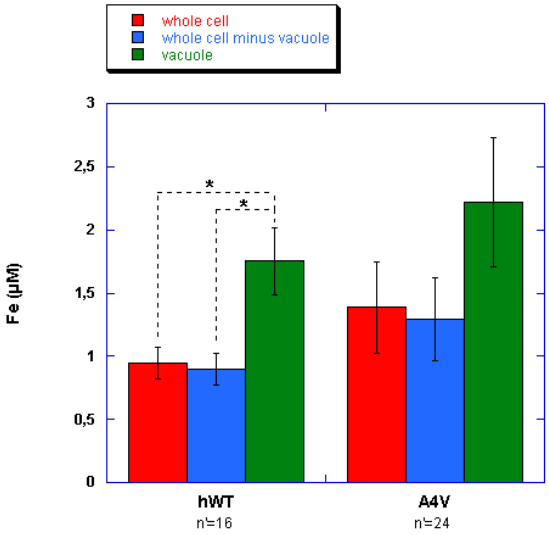

Figure 2. Iron concentrations (mean ± sd) in whole yeast, yeast minus vacuole and only vacuole.
n': total number of analyzed yeasts; Student's test (* P<0,05)

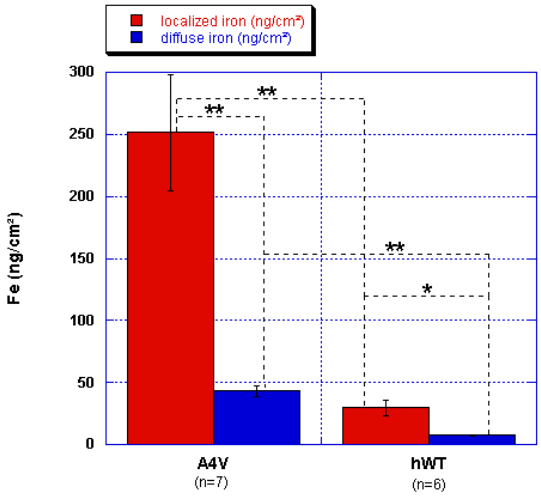

Figure 3. Iron concentrations(mean ± sd) in extra-vacuolar spots (*localized iron*) and in the rest of the cell (*diffuse iron*) for hWT and A4V yeast strains.
n: total number of analysis; Student's test (* P<0,05, ** P<0,01)