DNA damage repair ability in cell cycle arrested *Saccharomyces cerevisiae* cells

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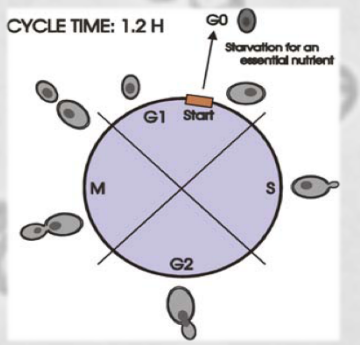
Introduction

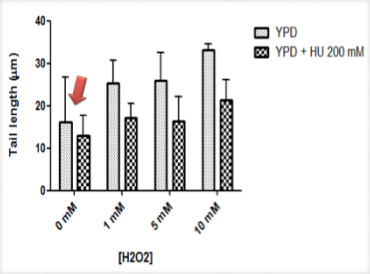
The present work investigates DNA damage repair ability in *Saccharomyces cerevisiae* cells (strain BY4741. *MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0)* arrested in the S phase of the cell cycle. Using the DNA replication blocking drug hydroxyurea (HU) and hydrogen peroxide as an oxidative damage causing agent, cells were analyzed by the comet assay, previously optimized for yeast in our laboratory. DNA damage and DNA damage repair capacity were evaluated. The tail length was chosen as a parameter for determination of the DNA damage extension.

Moreover, the effect of HU was investigated in *S. cerevisiae* cells pre-treated with *Ginkgo biloba* leaf extract (GBE). This extract was showed, by previous studies of our group, to have an antigenotoxic effect on cells exposed to oxidative stress and to improve DNA damage repair.

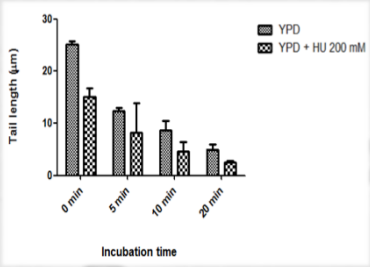
S. cerevisiae cell cycle



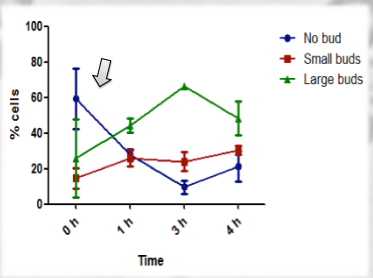
Results



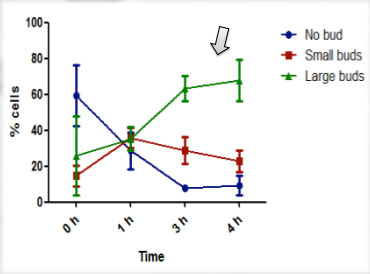
Treatment of *S.cerevisiae* cells with HU decreases DNA damage provoked by exposure to different concentrations of H_2O_2.



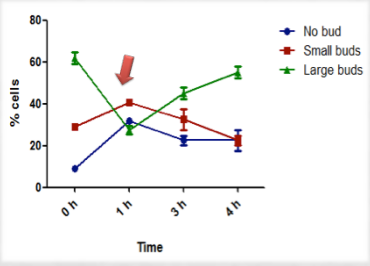
Incubation at 37ºC allows DNA damage repair in yeast cells grown on YPD and YPD+HU (200 mM) medium, after an exposure to oxidative shock by 10 mM H_2O_2.



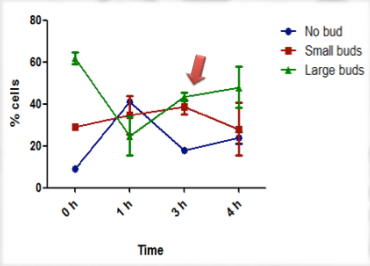
Budding index of *S. cerevisiae* cell culture, grown on YPD medium along 4 hours after pre-inoculum dilution (0h corresponds to undiluted culture). During the first hour, a decrease of unbudded cells and an increase of budding cells were observed.



Budding index of *S. cerevisiae* cell culture, grown on YPD+HU medium along 4 hours after pre-inoculum dilution (0h corresponds to undiluted culture immediatly before HU addition). As expected, HU causes cell cycle arrest (observed as an increase of non-dividing, large-budded cells).



Budding index of *S. cerevisiae* cell culture, grown on YPD medium for approximately 18 hours (0h corresponds to the culture immediately before addition of fresh medium).



Budding index of *S. cerevisiae* cell culture, grown on YPD medium for approximately 18 hours. At the time point of 0h, GBE was added. Cell cycle delay was observed (as an increase of small-budded cells), suggesting a transient arrest at an early stage of S phase).

Concluding remarks

✓ The results of yeast comet assay suggest the presence of the protective effect of HU towards oxidative DNA damage provoked by H_2O_2.
✓ GBE appears to delay yeast cell cycle, causing an increase of small-budded cells and a transient arrest at an early S phase, showing protective as HU.
✓ Cell cycle arrest provoked by GBE, permits targeting of yeast cell resources for DNA damage repair, supporting the GBE antigenotoxic effect reported previously by our group (Marques, F. 2009. Master thesis; see also poster #P139).