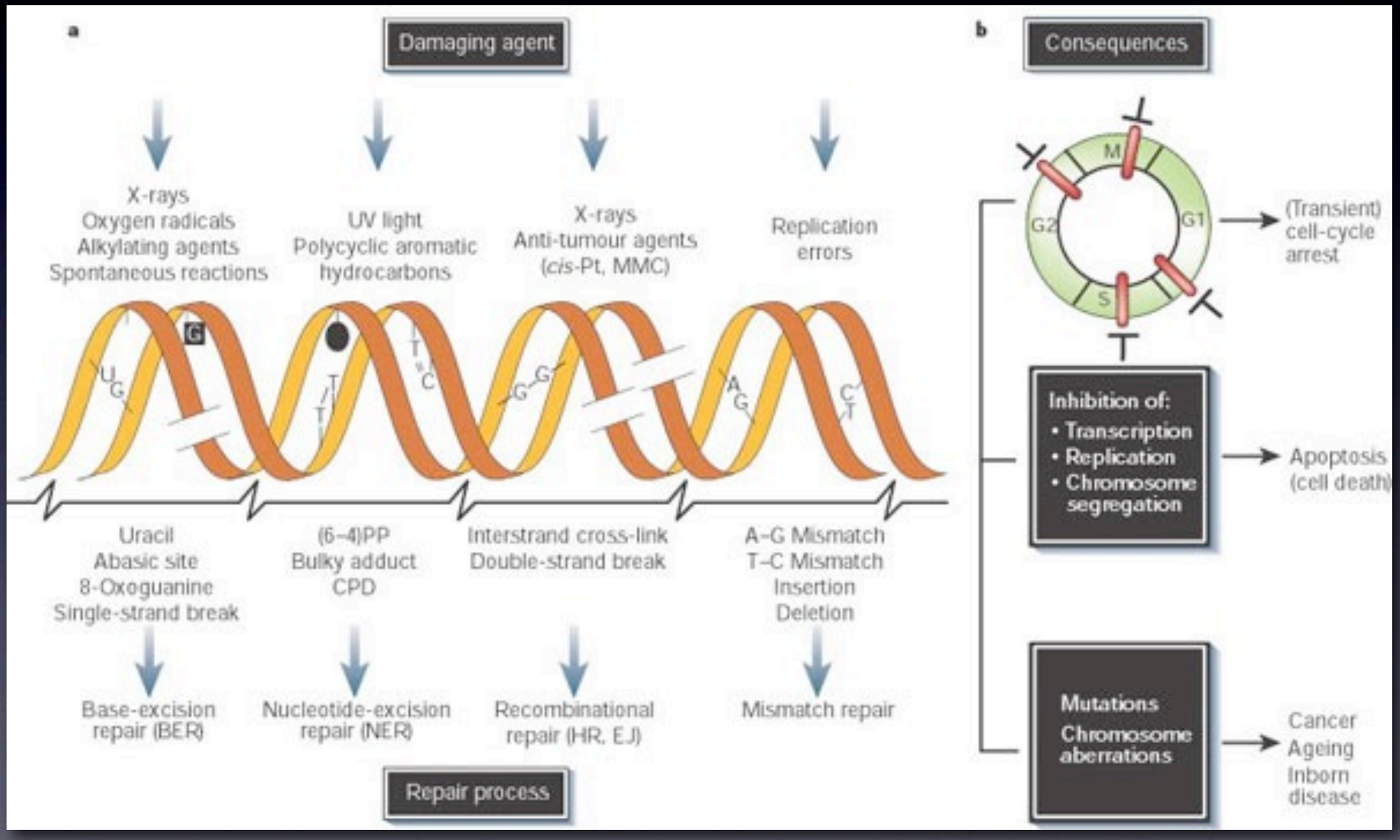
Analysis of DNA damage and repair in *Saccharomyces cerevisiae* using the comet assay in the characterization of antigenotoxicity of plant extracts and phytochemicals

Bioplant
Programa Inter-Universitário de Doutoramento
MAP

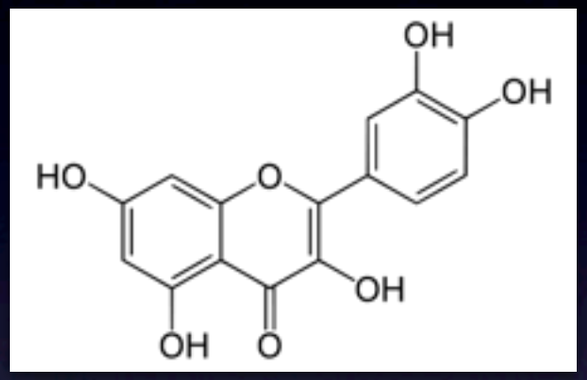
2nd Workshop BioPlant UMinho, Braga, 2011

DNA damage and repair

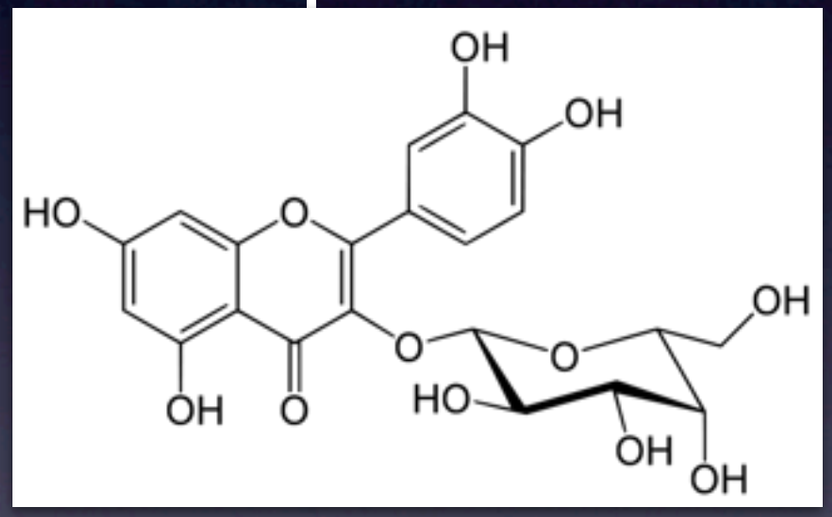


Flavonoids and antigenotoxicity

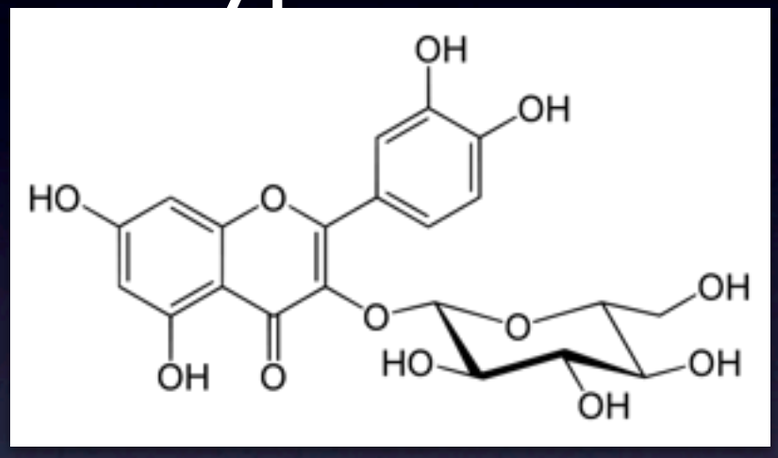
Quercetin



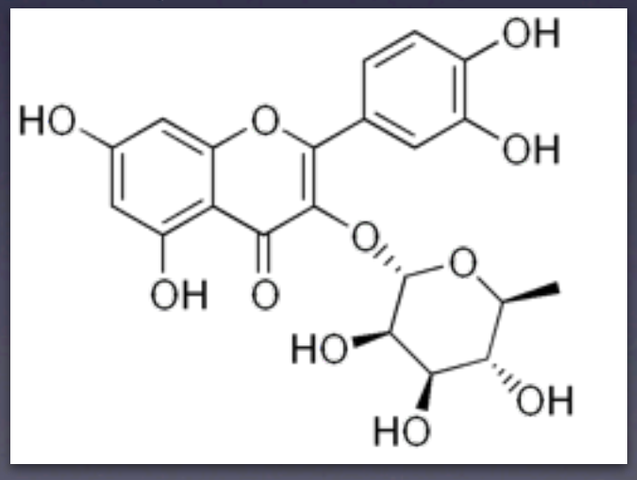
Isoquercitrin



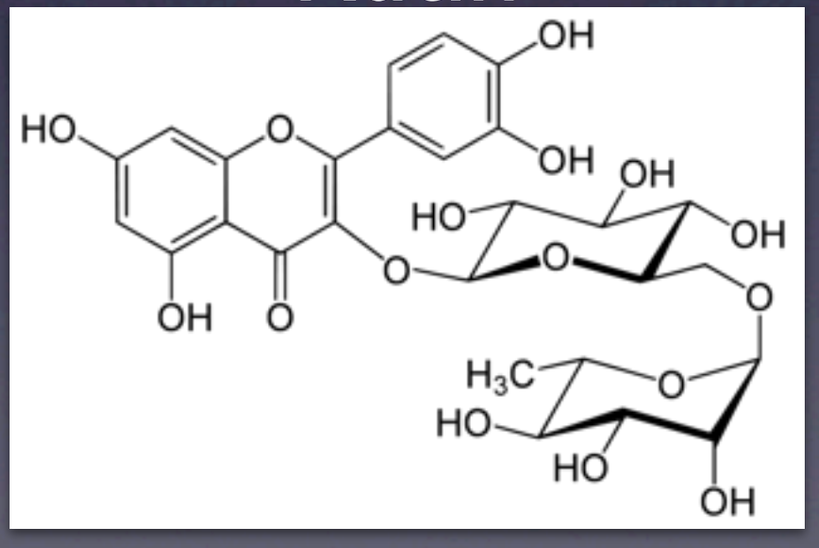
Hyperoside



Quercitrin



Rutin



It must be great to work with yeast!

- The first eukaryotic organism with the genome sequenced
- The first microarray experiments were made with yeast

- Large scale analyses are “easy” with yeasts:
  - Euroscarf project
  - Protein interactions
  - Protein localization

Fundamental mechanisms are highly conserved

DNA damage checkpoint proteins

	Budding yeast	Fission yeast	Human
PIKK	Mec1	Rad3	ATR
PIKK	Tel1	Tel1	ATM
Adaptor	Rad9	Crb2	53BP1, MDC1, BRCA1?
Rfc1 homolog	Rad24	Rad17	Rad17
9-1-1 clamp	Rad17	Rad9	Rad9
	Mec3	Hus1	Hus1
	Ddc1	Rad1	Rad1
MRX complex	Mre11	Mre11	Mre11
	Rad50	Rad50	Rad50
	Xrs2	Nbs1	Nbs1
BRCT domain adaptor?	Dpb11	Rad4/Cut5	TopBP1
Signaling kinase	Rad53	Cds1	Chk2
Signaling kinase	Chk1	Chk1	Chk1
Polo kinase	Cdc5	Plo1	Plk1
Securin	Pds1	Cut2	Securin
Separase	Esp1	Cut1	Separase
APC-targeting subunit	Cdc20	Slp1	$p55^{CDC}$/CDC20

Harrison and Haber. 2006. *Annu Rev Genet* 40:209-35

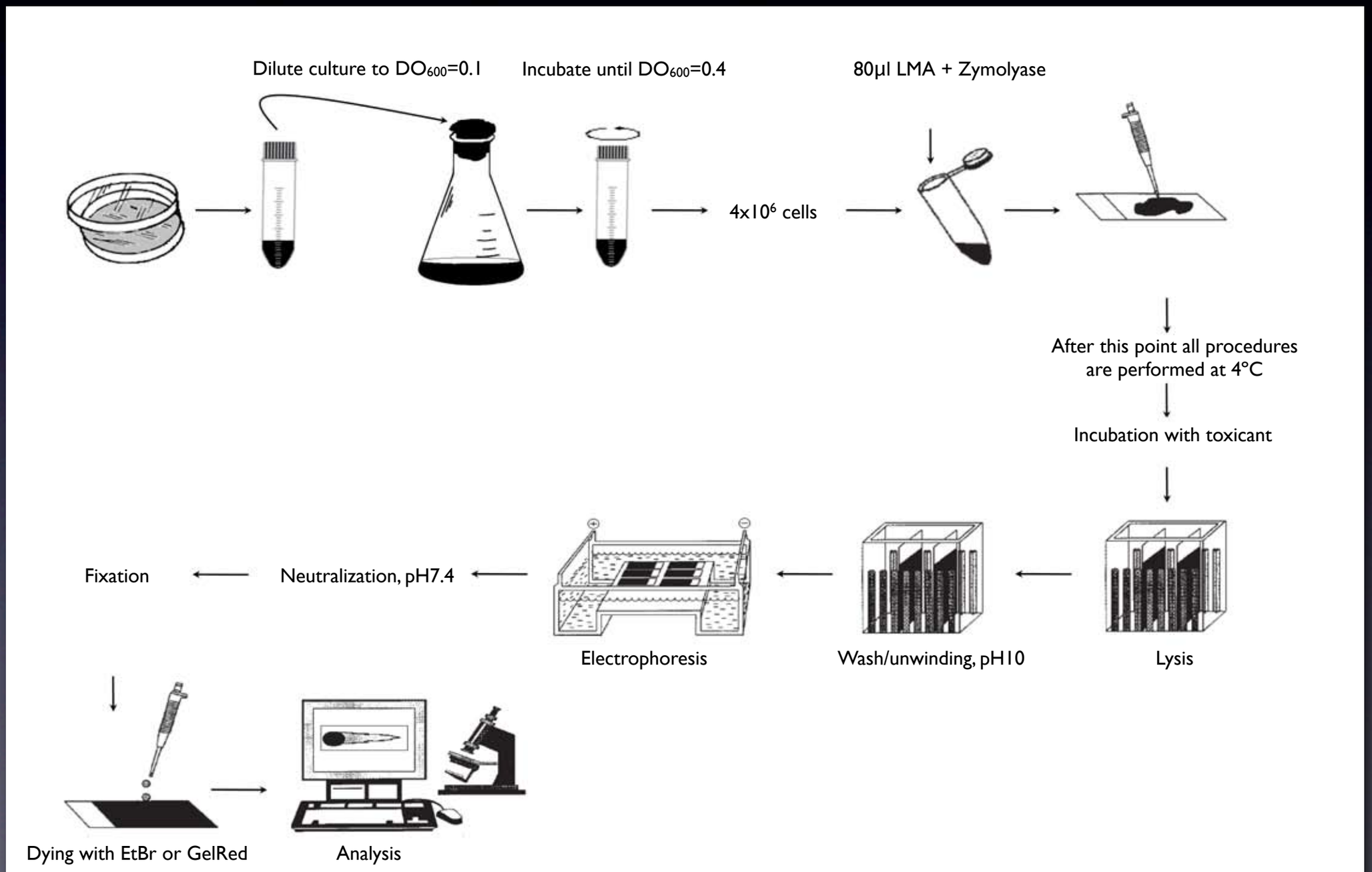
NER gene orthologs in selected eukaryotes[a]

Human	*S. pombe*	*S. cerevisiae*	*Drosophila*
XPC	*rhp41, rhp42*	*RAD4*	*mus210*
RAD23B (HR23B), RAD23A (HR23A)	*rhp23*	*RAD23*	*Rad23*
XPA	*rhp14*$^+$	*RAD14*	*Xpac*
RPA1	*ssb1*$^+$	*RFA1*	*RpA-70*
RPA2	*ssb2*$^+$	*RFA2*	*RpA-30*
RPA3	*ssb3*$^+$	*RFA3*	*RpA-8*
XPG (ERCC5)	*rad13*$^+$	*RAD2*	*mus201*
ERCC1	*swi10*$^+$	*RAD10*	*Ercc1*
XPF (ERCC4)	*rad16*$^+$ (*swi9*$^+$, *rad10*$^+$, *rad20*$^+$)	*RAD1*	*mei9*
LIG1	*cdc17*$^+$	*CDC9*	*DNA-lig1*
CSA (CKN1)			—
CSB (ERCC6)		*RAD26*	—
DDB1 (p127)	*ddb1*$^+$	—	*Ddb1*
DDB2 (XPE; p48)	—	—	—
MMS19L (MMS19)		*MMS19*	*Mms19*
—	—	*RAD7*	—
—	*rhp16*$^+$	*RAD16*	—
TFIIH subunits			
XPB (ERCC3)	*ercc3sp*$^+$	*SSL2 (RAD25)*	*hay (haywire)*
XPD (ERCC2)	*rad15*$^+$ (*rad5*$^+$, *rhp3*$^+$)	*RAD3*	*Xpd*
GTF2H1 (p62)	*tfb1*$^+$	*TFB1*	*Tfb1*
GTF2H2 (p44)	*ssl1*$^+$	*SSL1*	*Ssl1*
GTF2H3 (p34)	*Tfb4*$^+$	*TFB4*	*Tfb4*
GTF2H4 (p52)	*Tfb2*$^+$	*TFB2*	*Tfb2*
GTF2H5 (TTDA)		*TFB5*	
CDK7	—	*KIN28*	*Cdk7*
CCNH	—	*CCL1*	*CycH*
MNAT1 (Mat1)	—	*TFB3*	*Mat1*

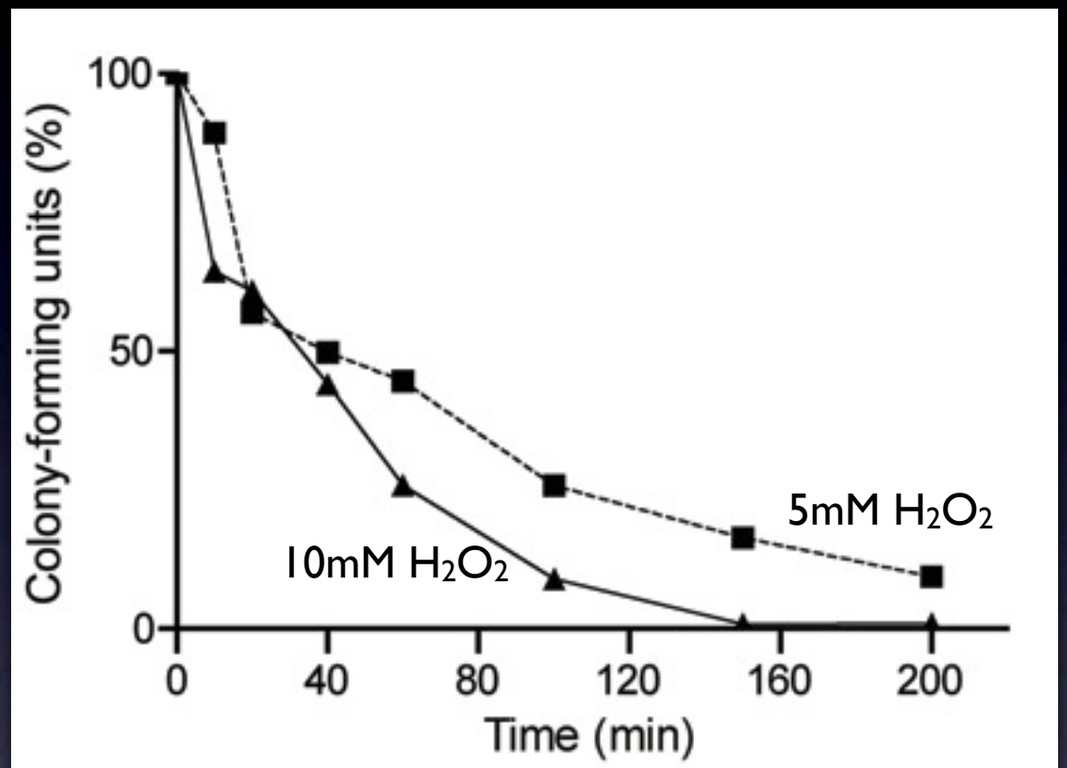
[a]—, no obvious ortholog is present in the genome. Some synonyms for gene names are shown in parentheses.

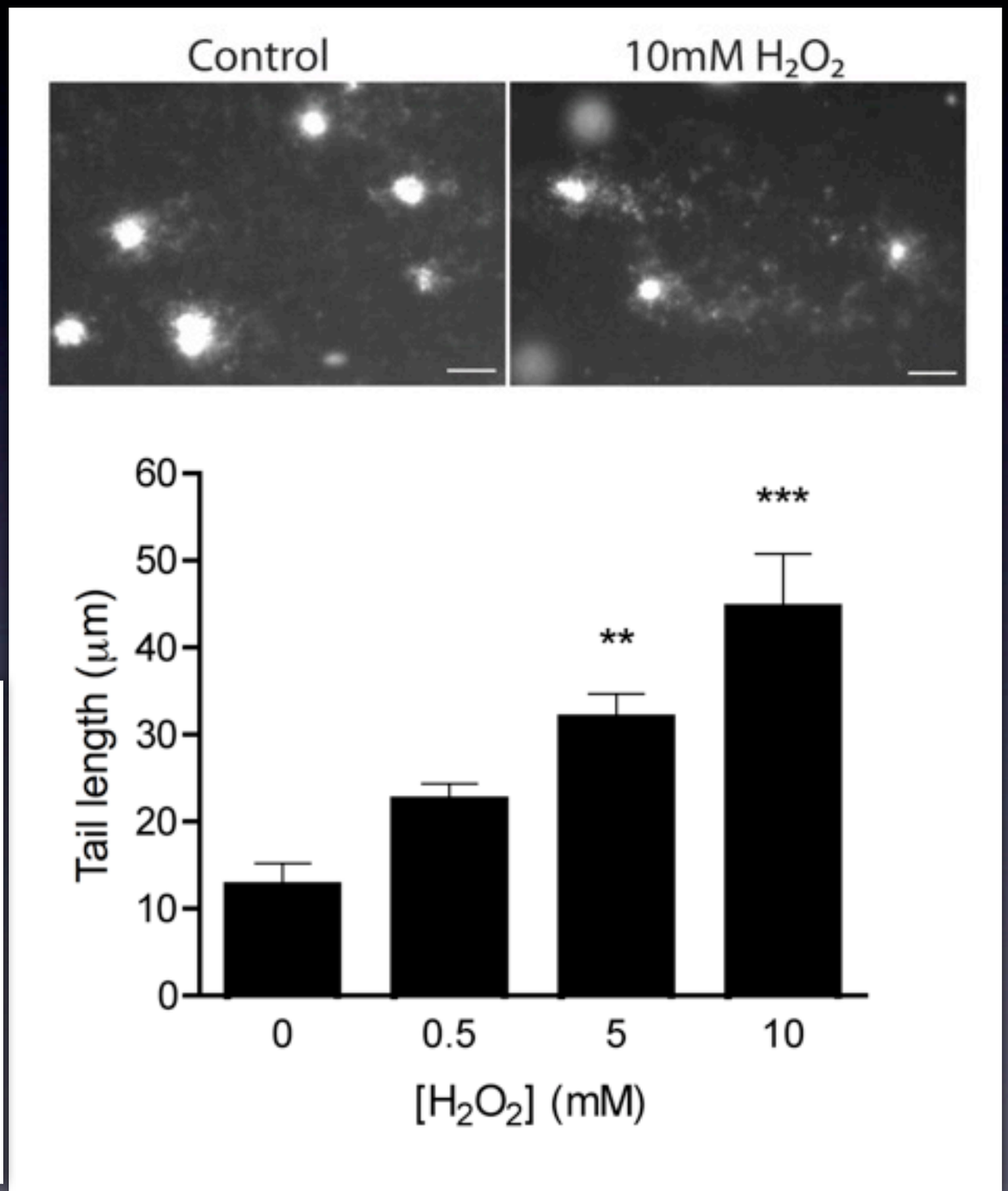
Friedberg *et al.* 2006. *DNA Repair and Mutagenesis, 2nd ed* ASM Press

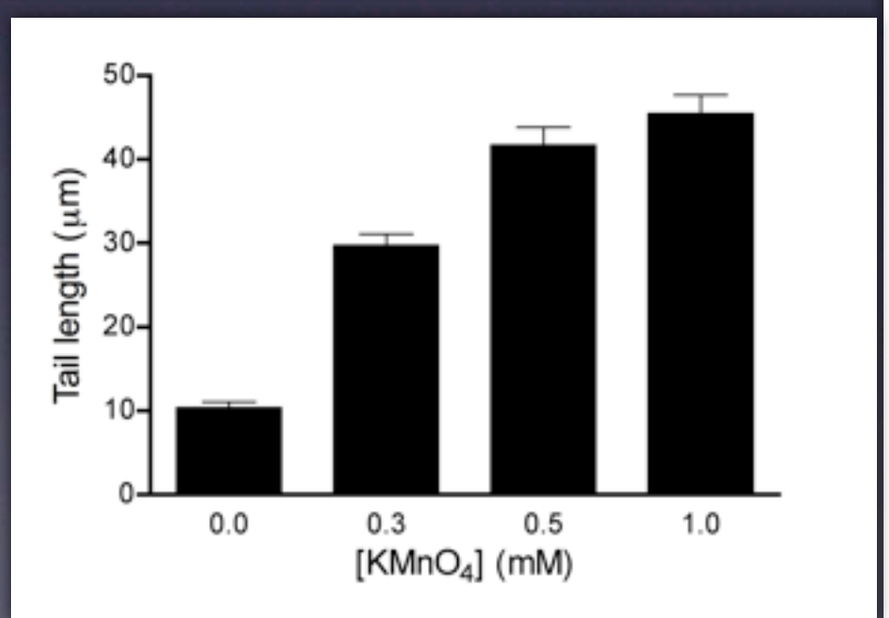
DNA damage assessment in yeast by the comet assay



DNA damage correlates with toxicant concentration

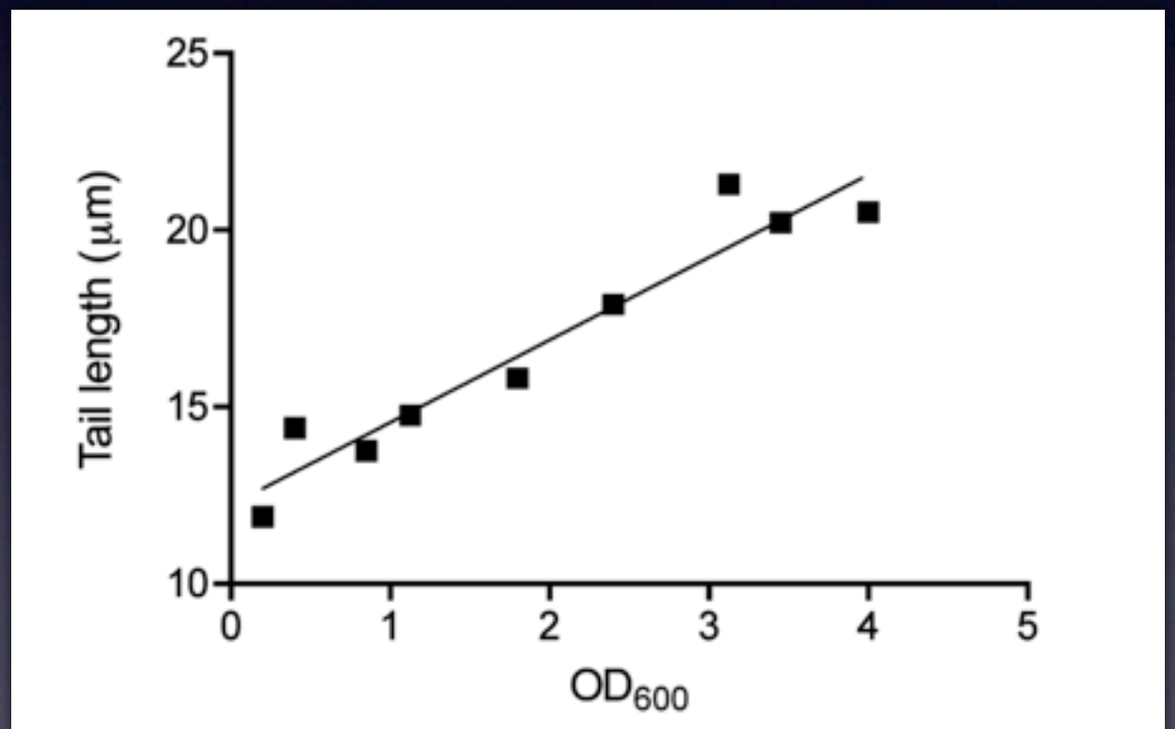


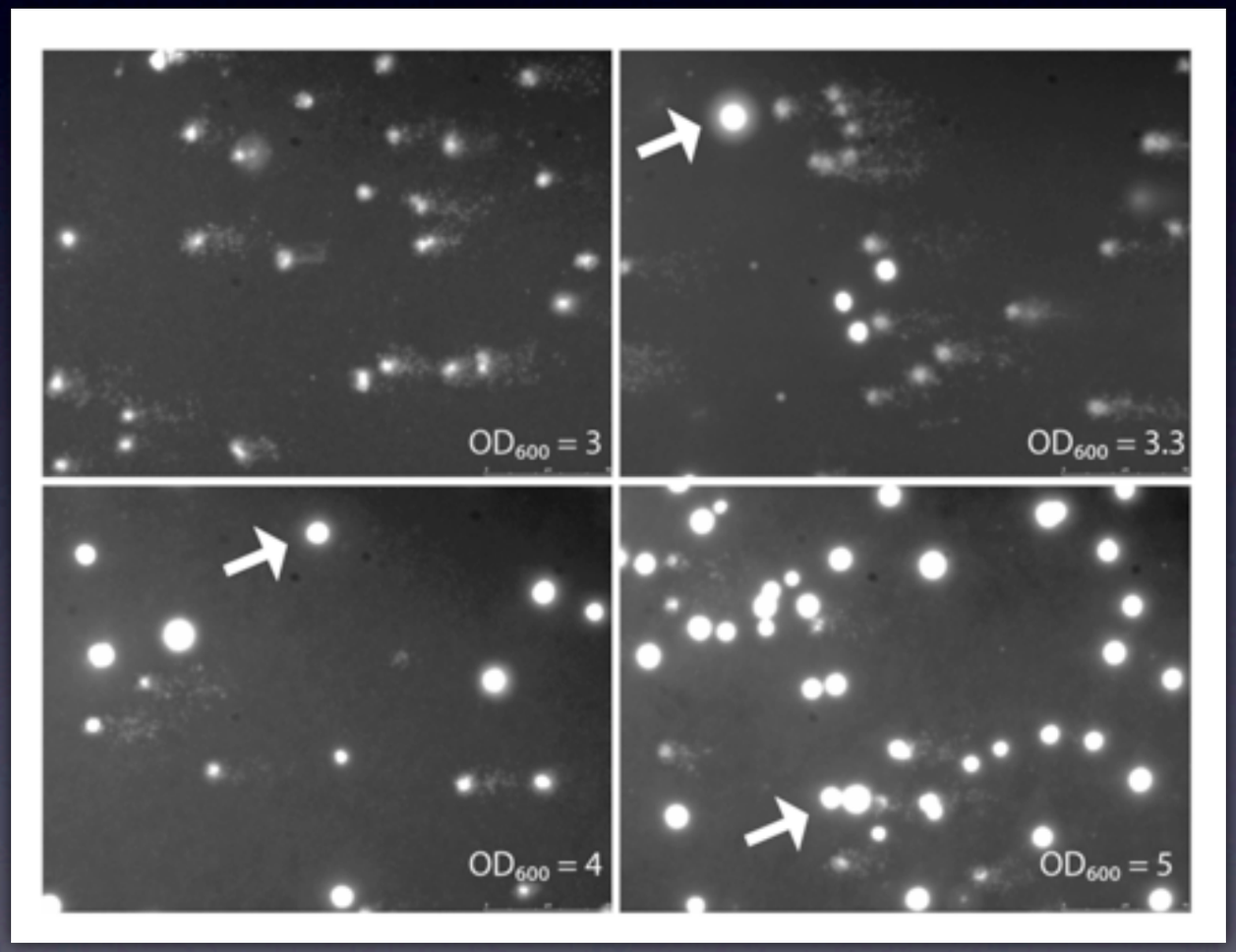




Azevedo *et al.*, 2011. *Yeast* 28:55-61

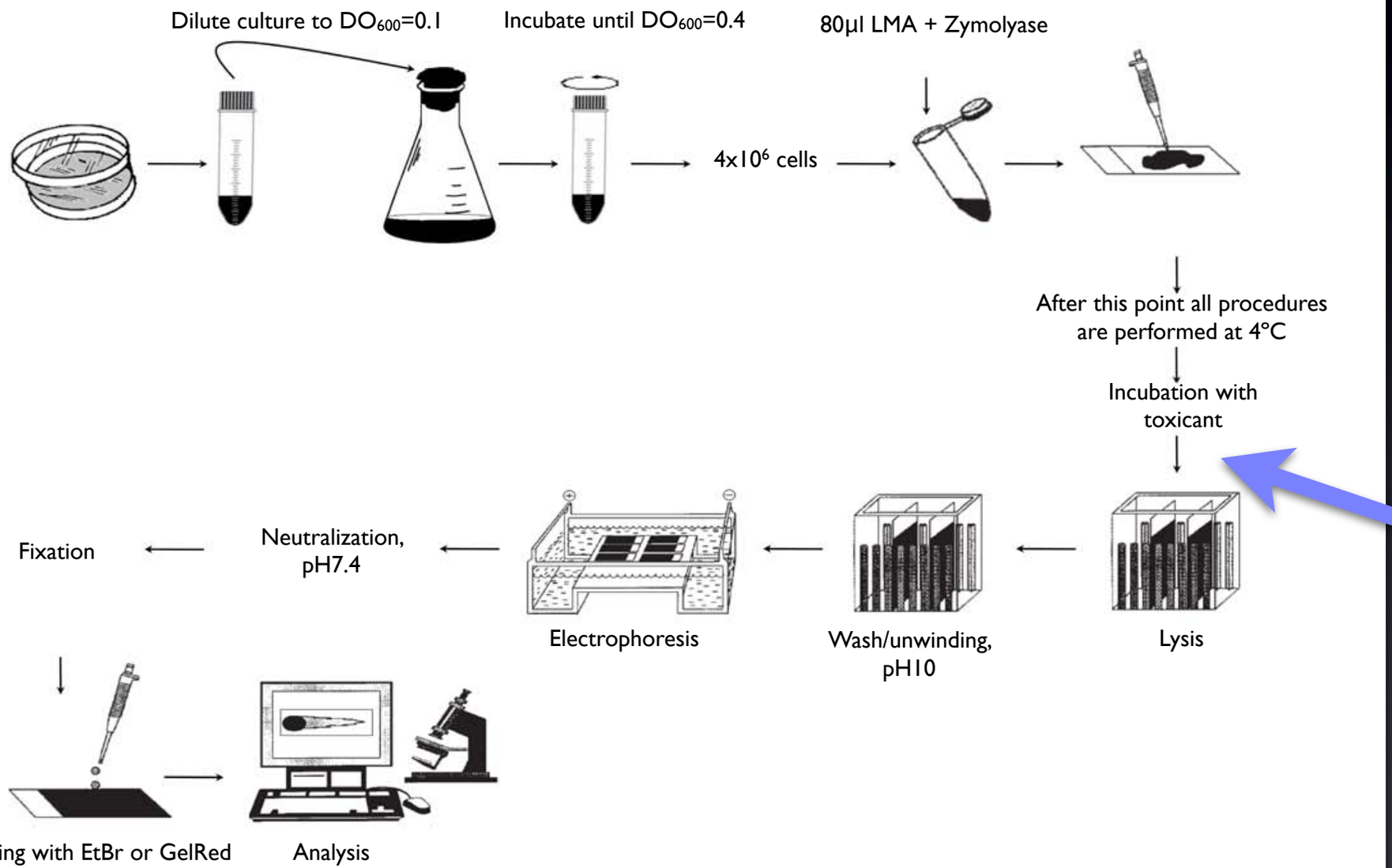
DNA damage increases along with culture aging





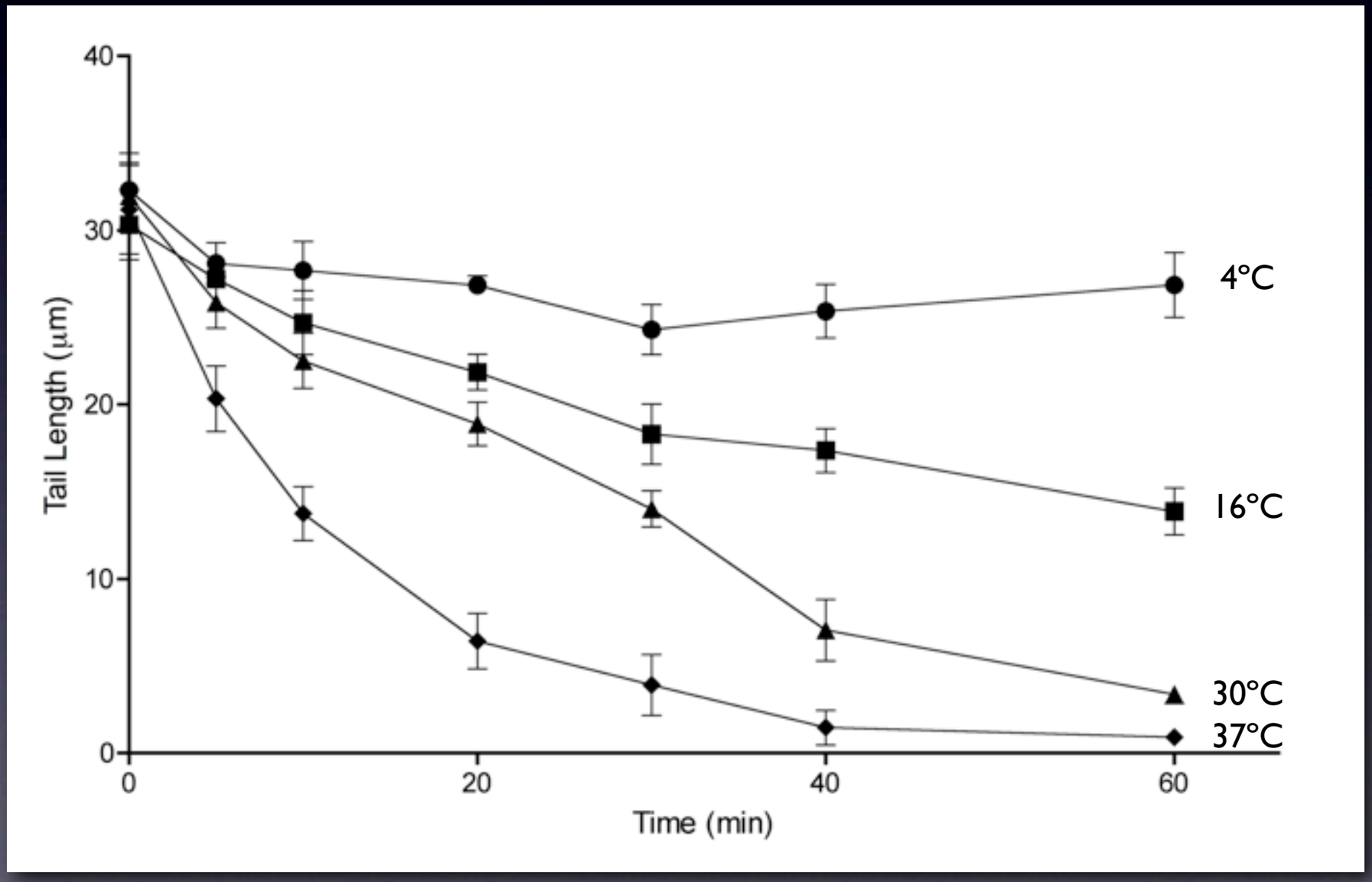
Azevedo *et al.*, 2011. *Yeast* 28:55-61

DNA damage repair evaluation by the comet assay



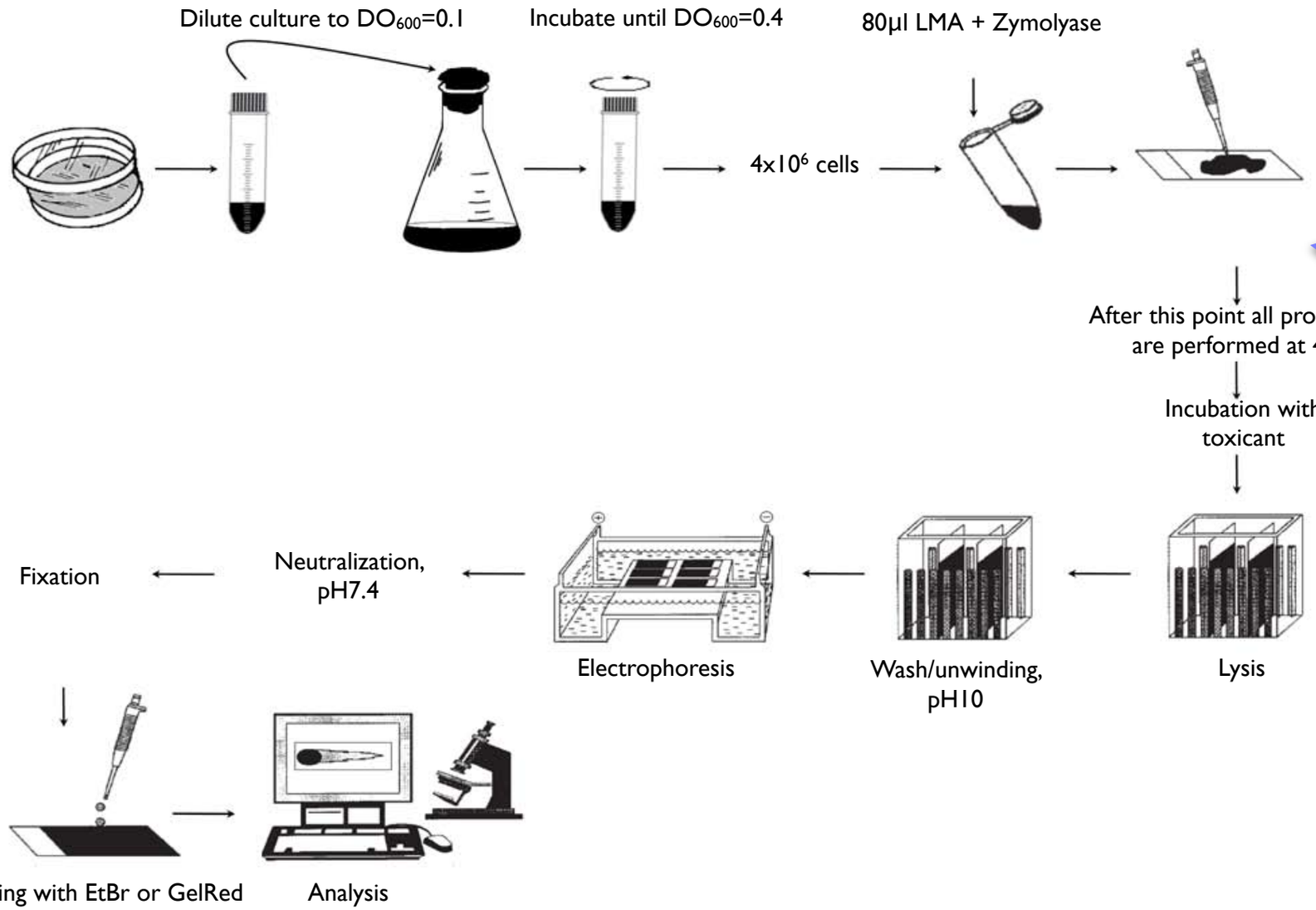
After removal of the toxic compound, cells are allowed to recover damage for different time points

DNA damage repair is abolished at 4°C



Azevedo *et al.*, 2011. *Yeast* 28:55-61

Antigenotoxicity evaluation by the comet assay

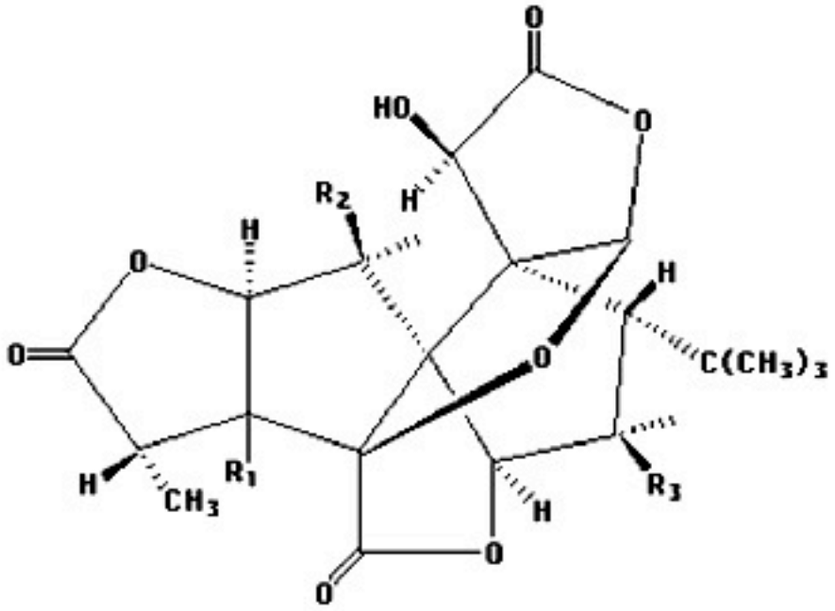


Incubation with natural compounds (pre-incubation with phytochemicals)

Ginkgo biloba, the atomic bomb survivor

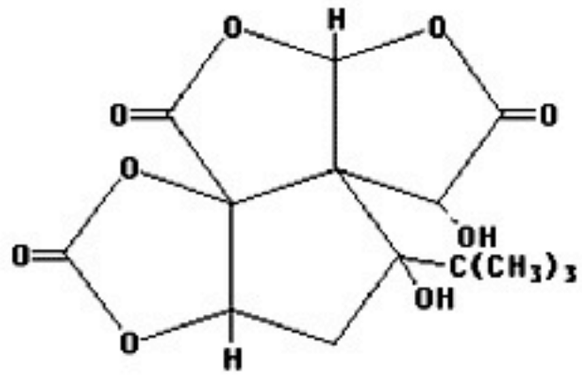
http://www.xs4all.nl/~kwanten/hiroshima.htm

Major Active Constituents of Ginkgo biloba

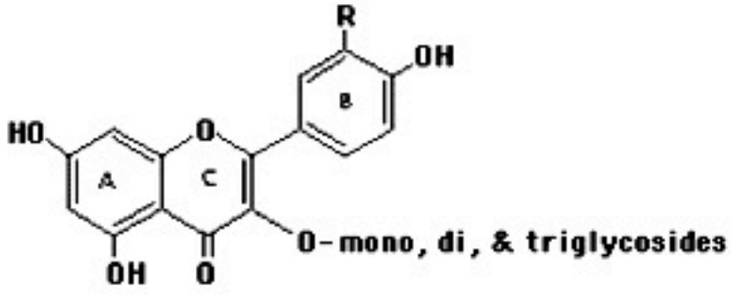


R1	R2	R3	Ginkgolide
OH	H	H	A
OH	OH	H	B
OH	OH	OH	C
H	OH	OH	M
OH	H	OH	J

Ginkgolide
(DITERPENES)

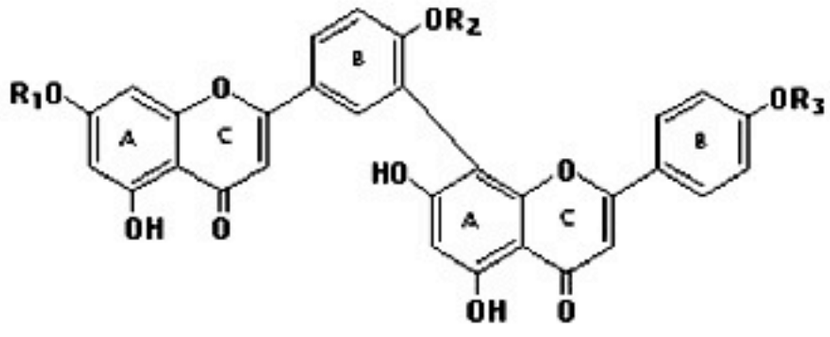


Bilobalide
(Sesquiterpene)



R	H	Kaempferol derivatives
R	OH	Quercetin derivatives
R	OCH3	Isorhamnetin derivatives

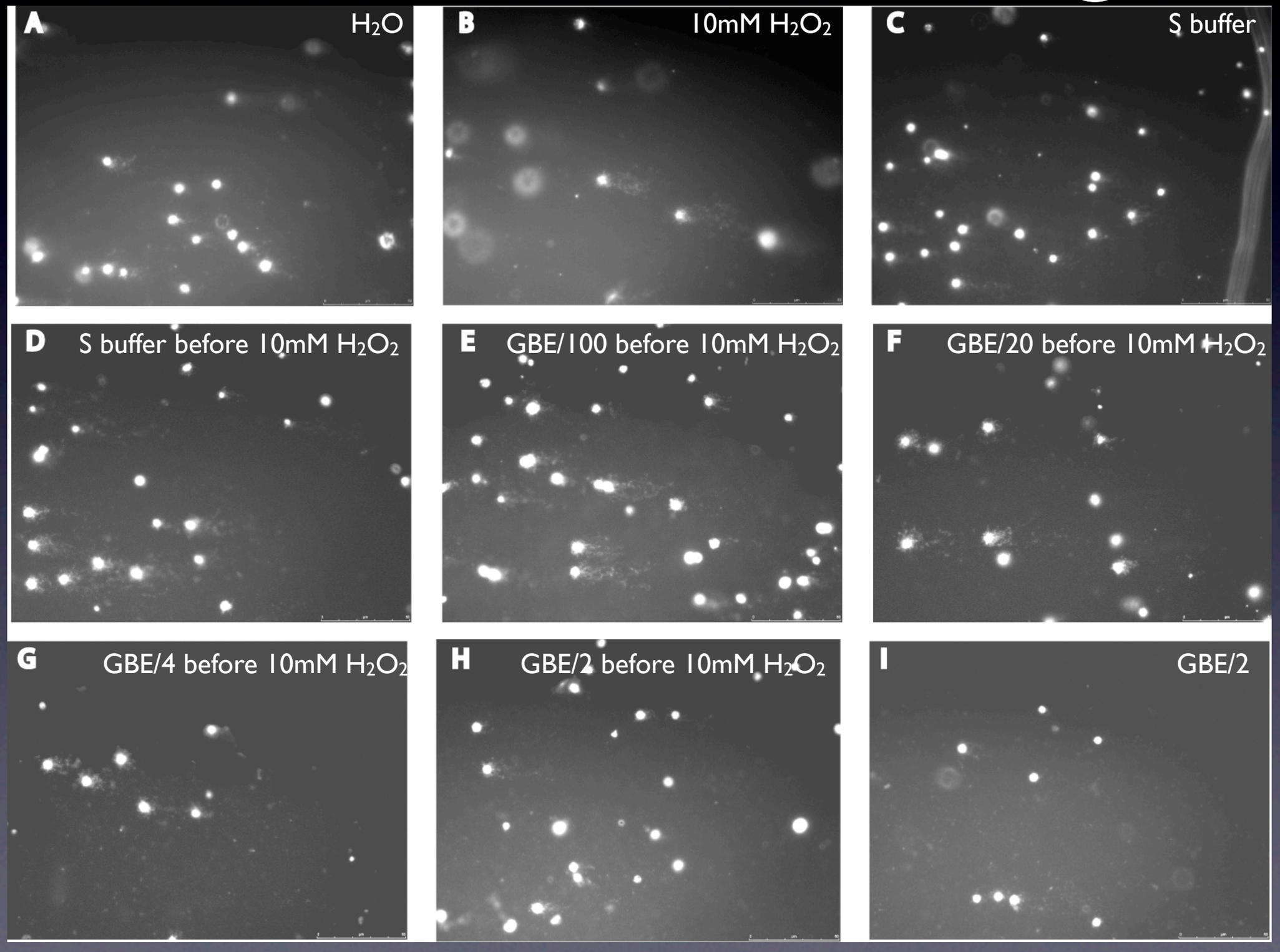
(all are flavonoid glycosides)



R1	R2	R3	Ginkgolide
H	H	H	Amentoflavone
H	CH3	H	Ailobetin
CH3	CH3	H	Ginkgetin
H	CH3	CH3	Isoginlgetin
CH3	CH3	CH3	Sciadopirysin

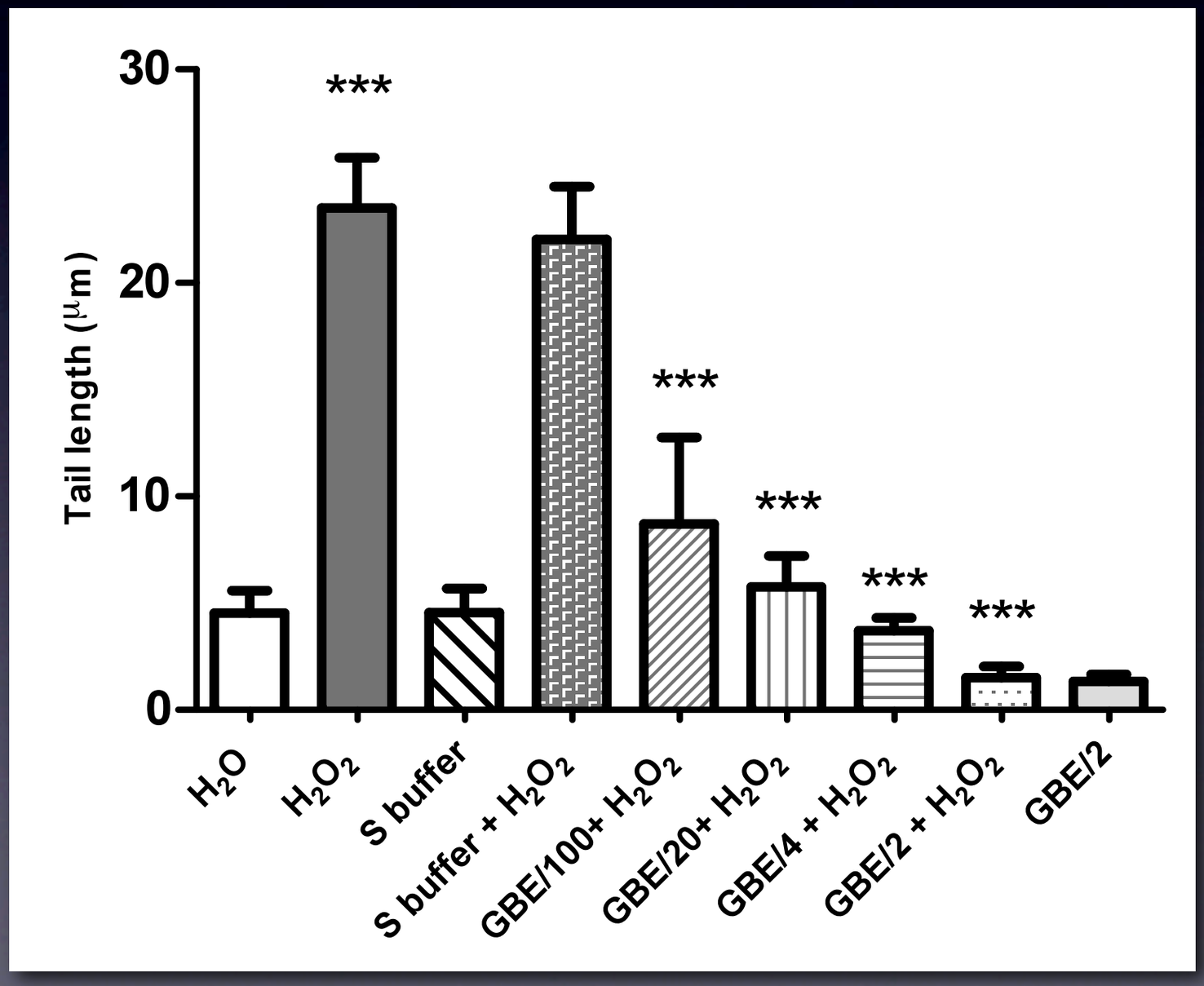
(all are biflavenoid derivatives)

GBE protects *S. cerevisiae* from oxidative DNA damage

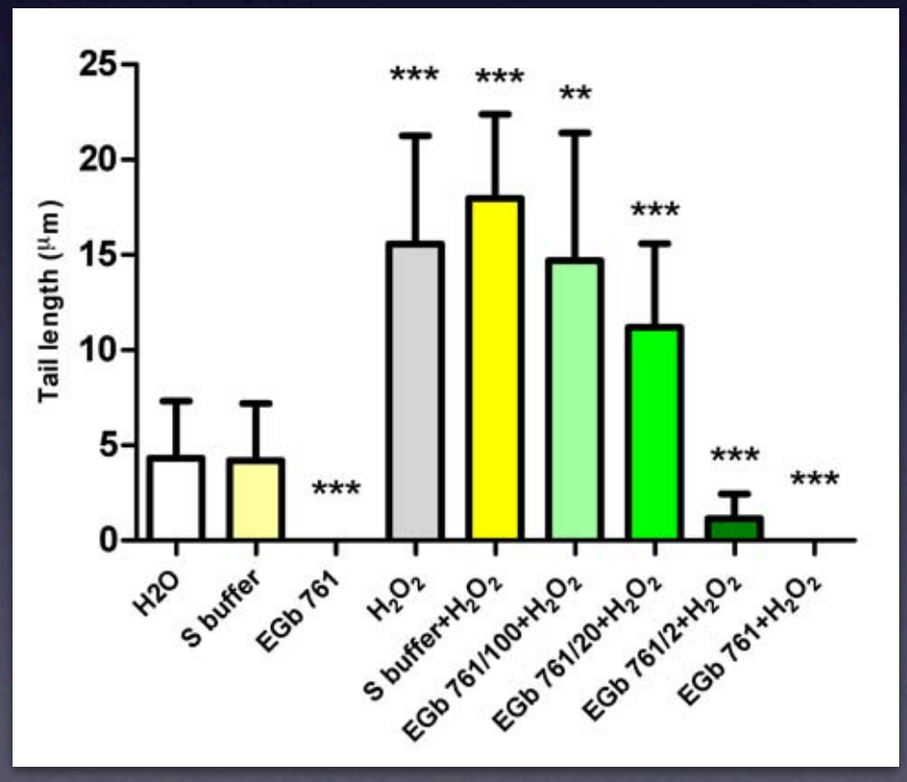


GBE protects *S. cerevisiae* from oxidative DNA damage

Pre-incubation with GBE

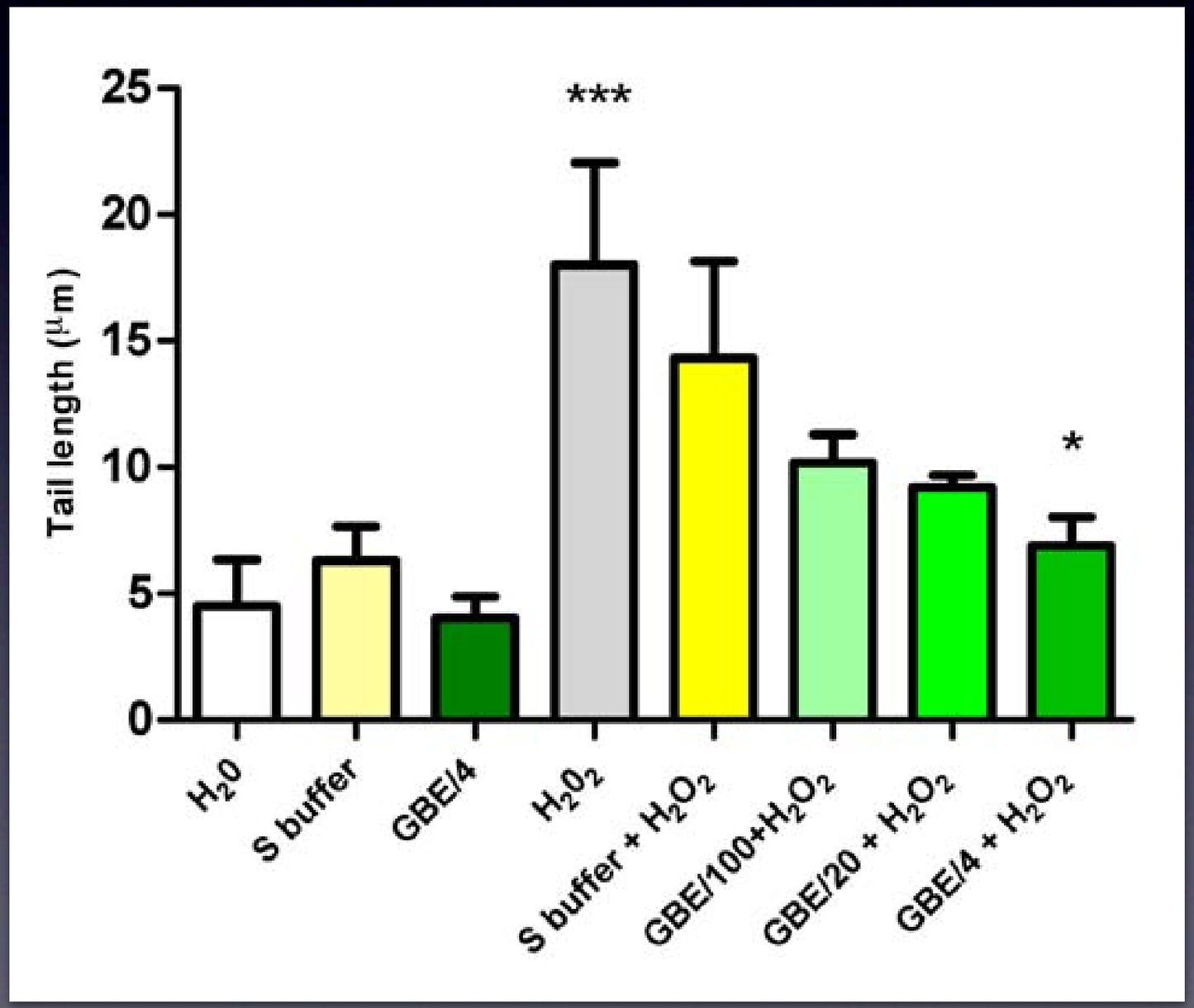


Assay with the standard extract EGb 761 (1mg/ml)



Marques *et al.*, 2011. *Food and Chemical Toxicology, in press*

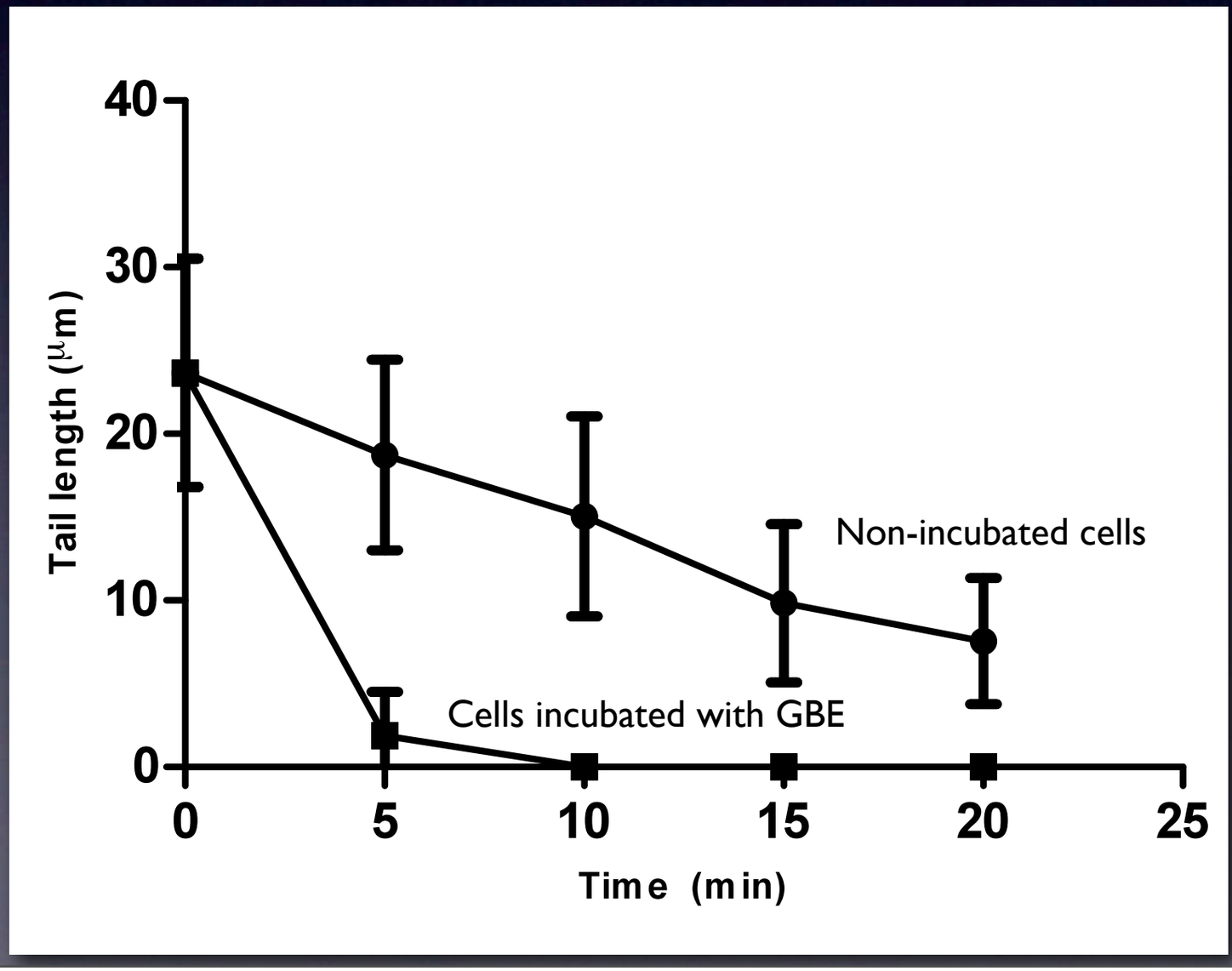
GBE also protects cells in co-incubation with H_2O_2



Marques *et al.*, 2011. *Food and Chemical Toxicology, in press*

GBE increases DNA repair capability upon oxidative damage

Post-incubation with GBE

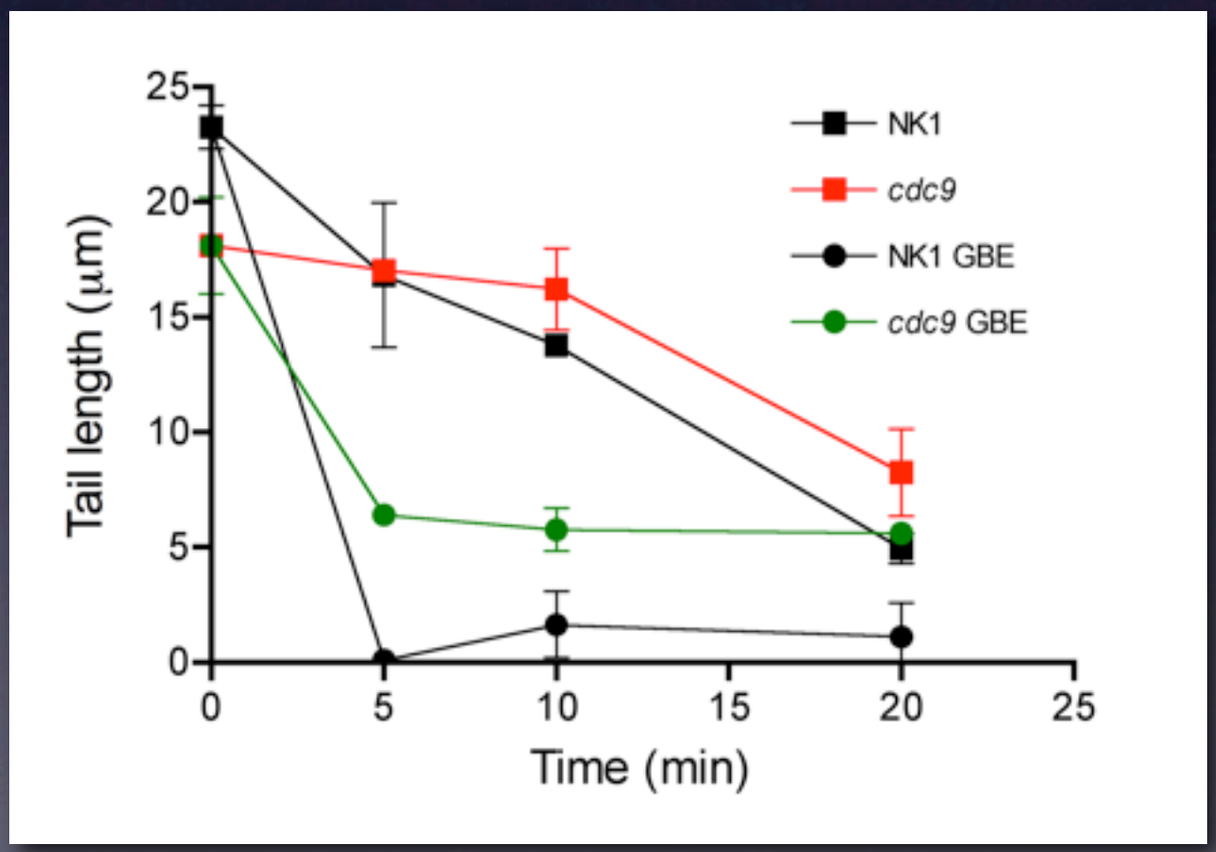


Marques *et al.*, 2011.
Food and Chemical Toxicology, in press

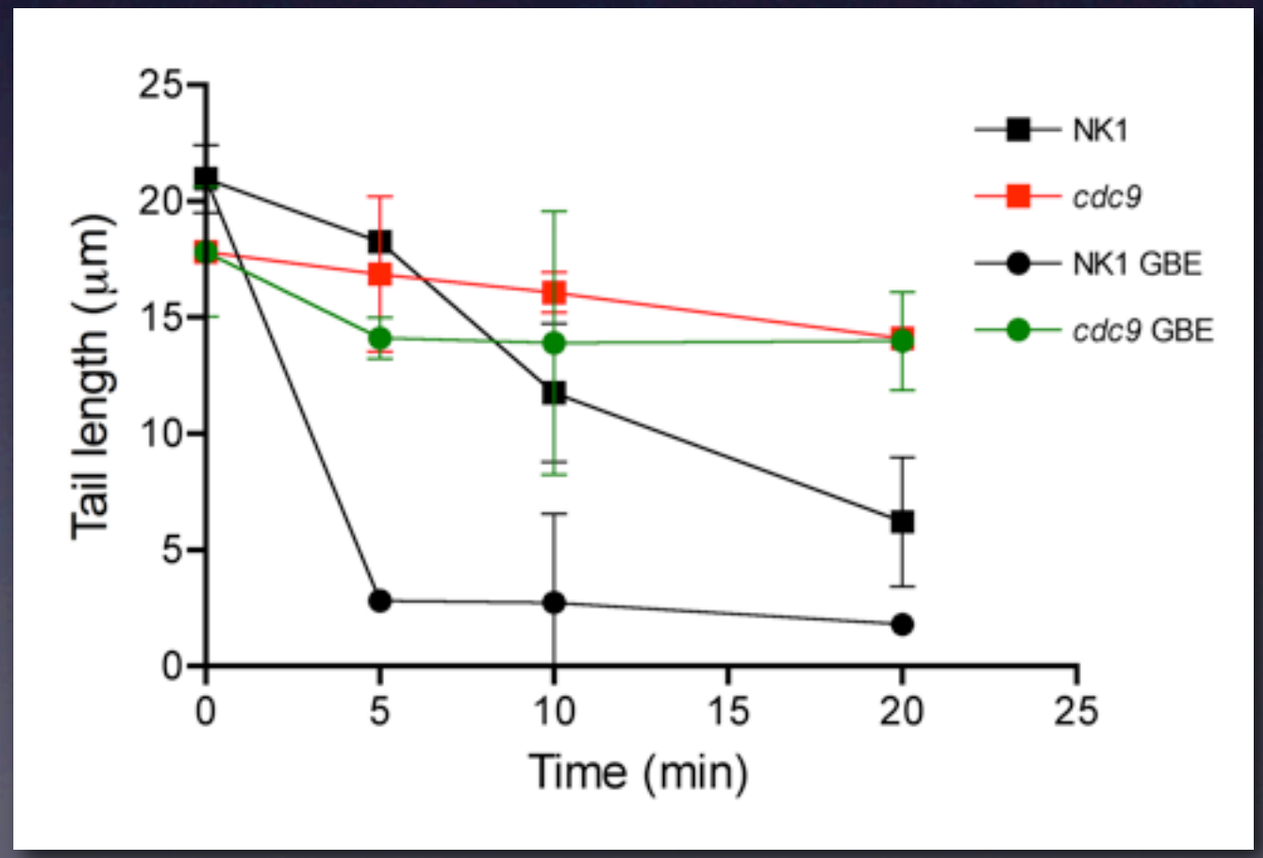
Yeast DNA repair defective mutants are insensitive to GBE

Cdc9: DNA ligase of the last step of NER and BER

23°C

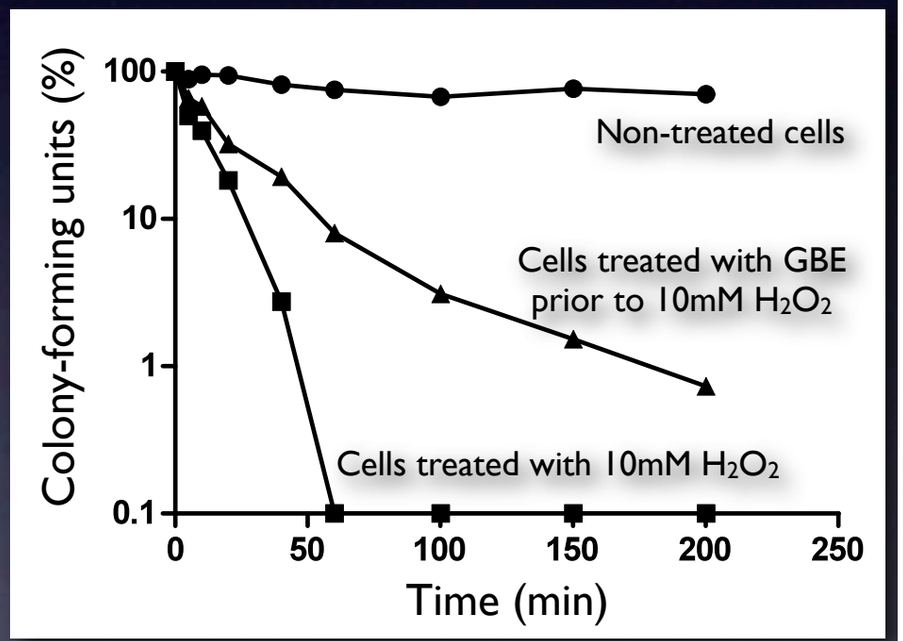


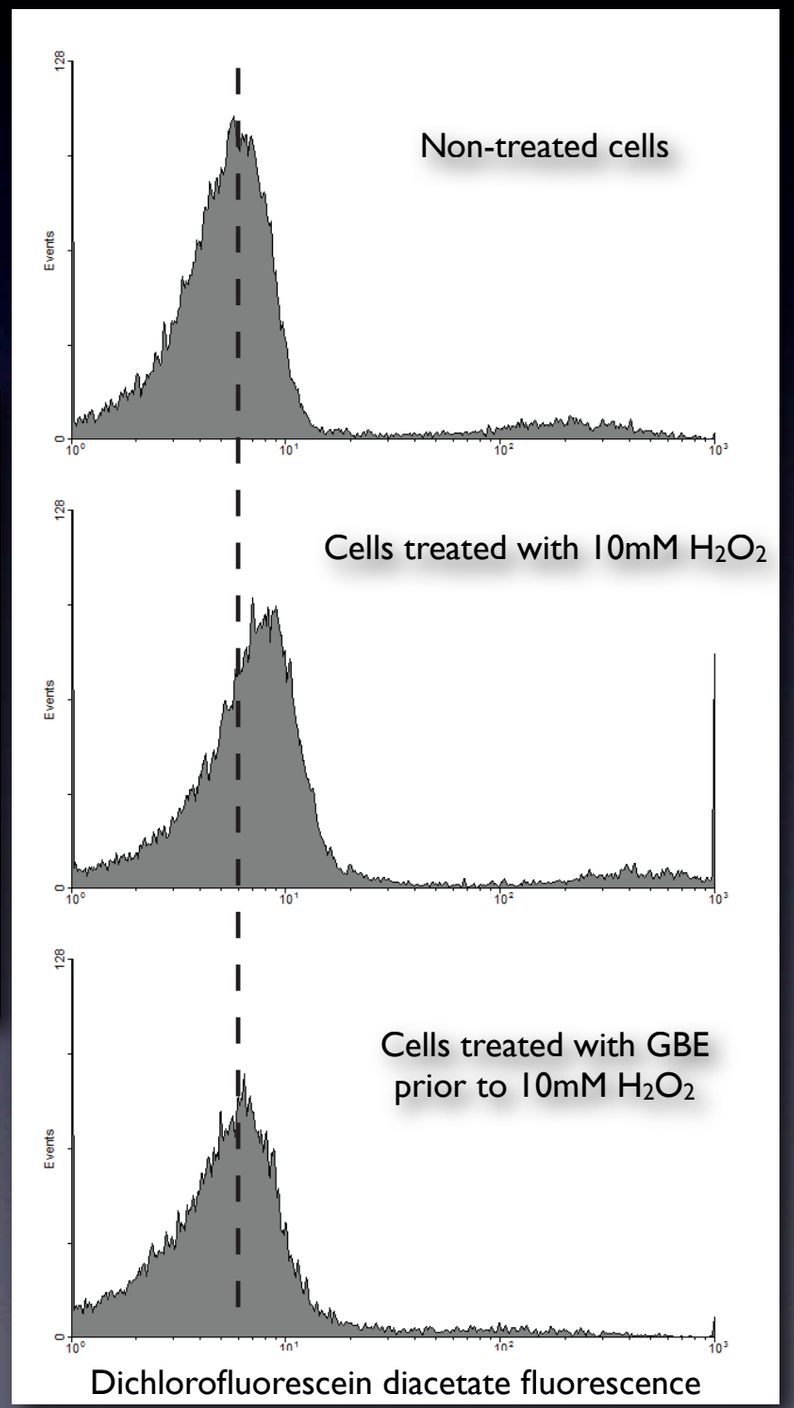
37°C

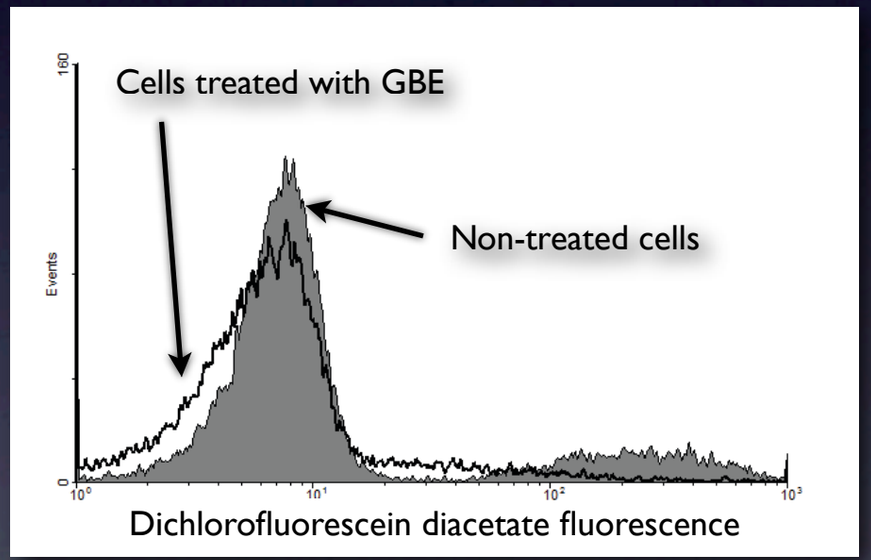


Marques *et al.*, 2011. *Food and Chemical Toxicology, in press*

GBE decreases intracellular oxidation

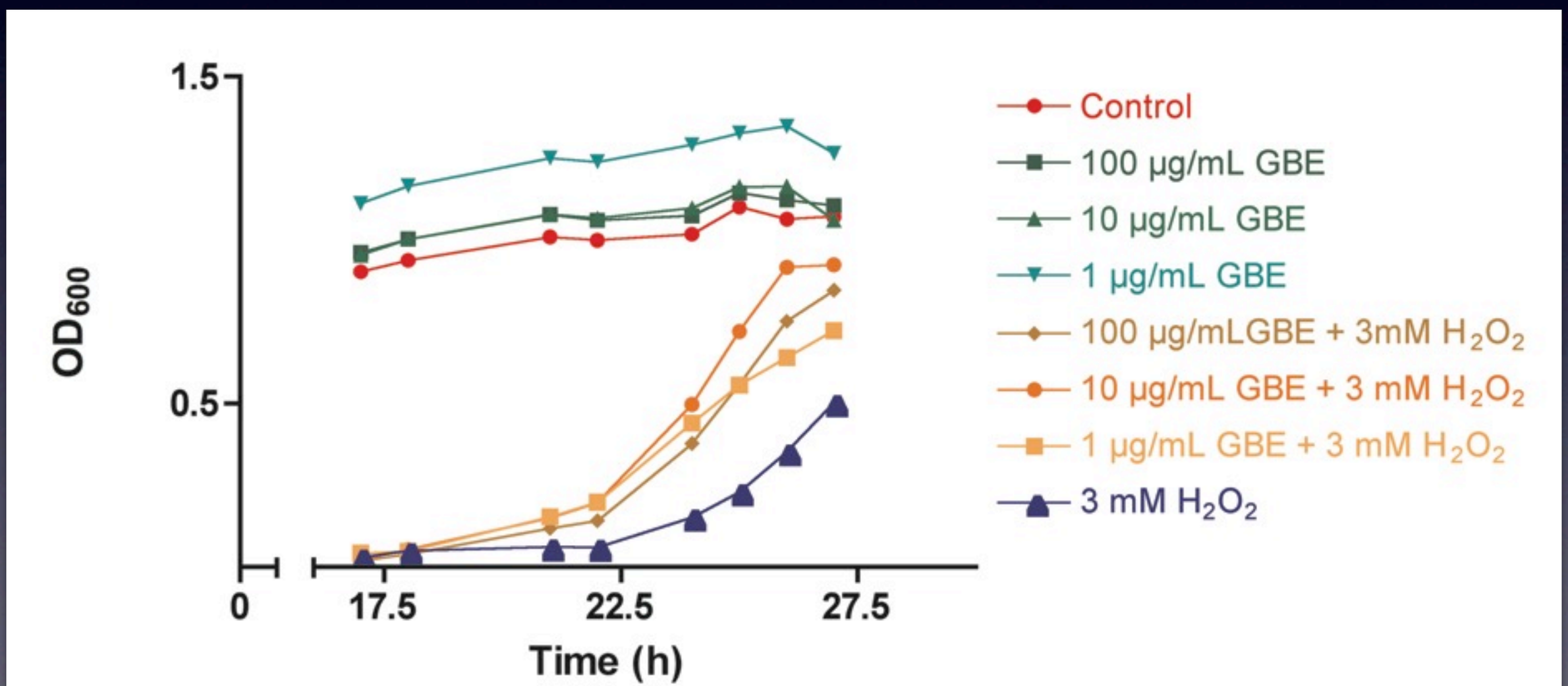




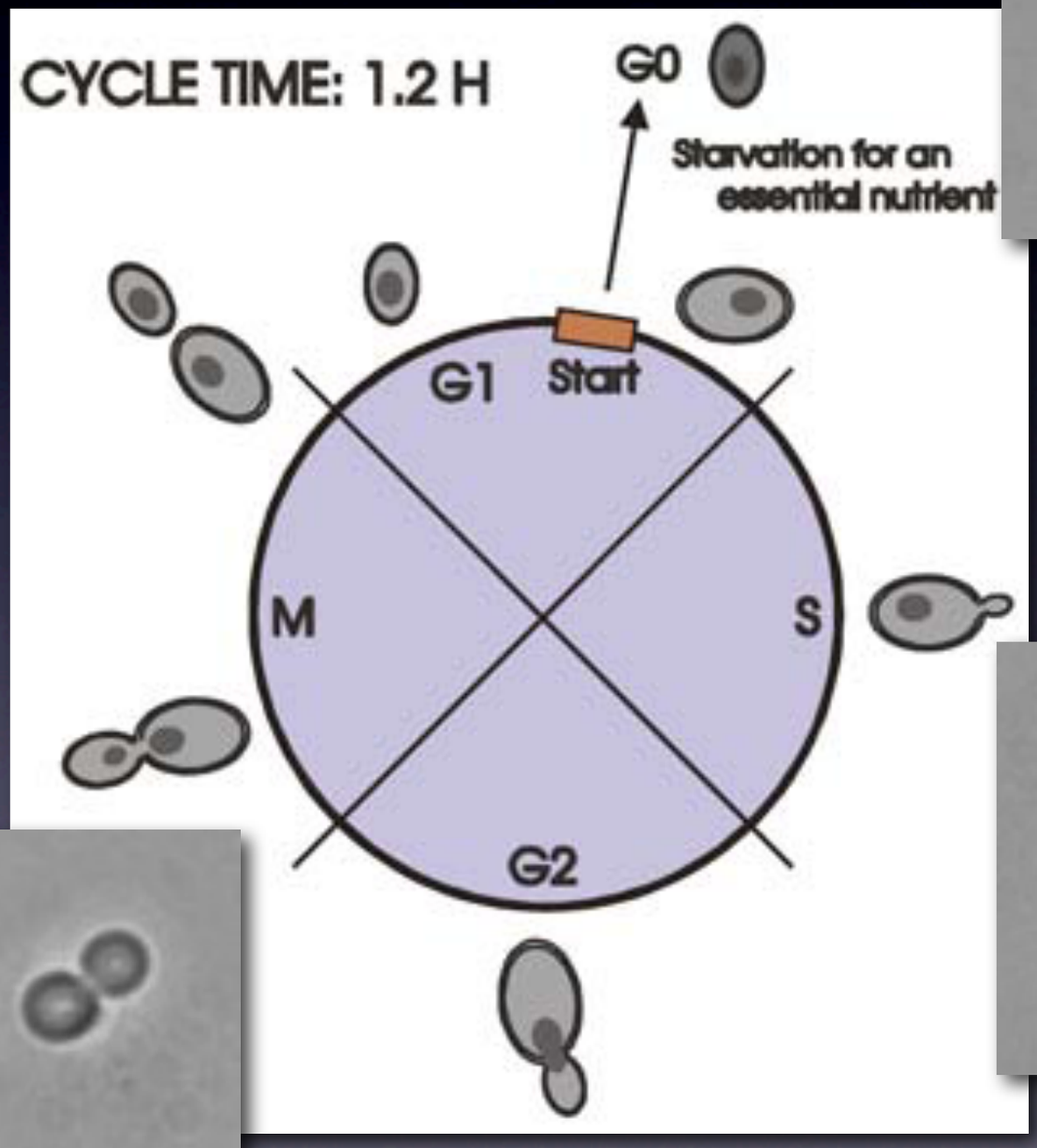


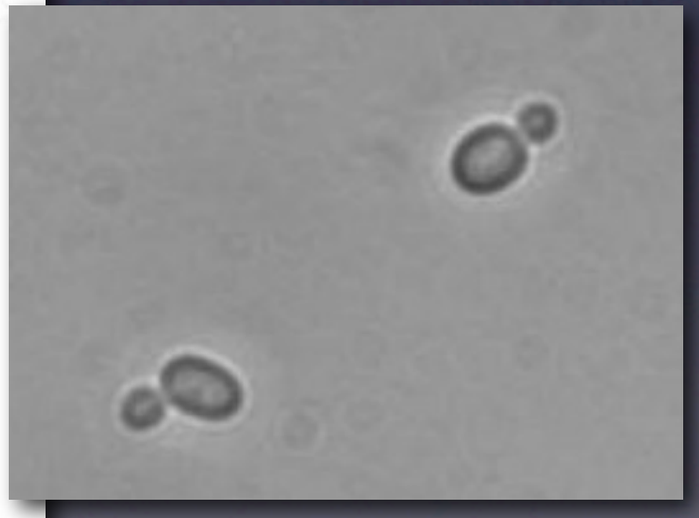
Marques *et al.*, 2011. *Food and Chemical Toxicology, in press*

Yeast cells have more fitness in the presence of GBE

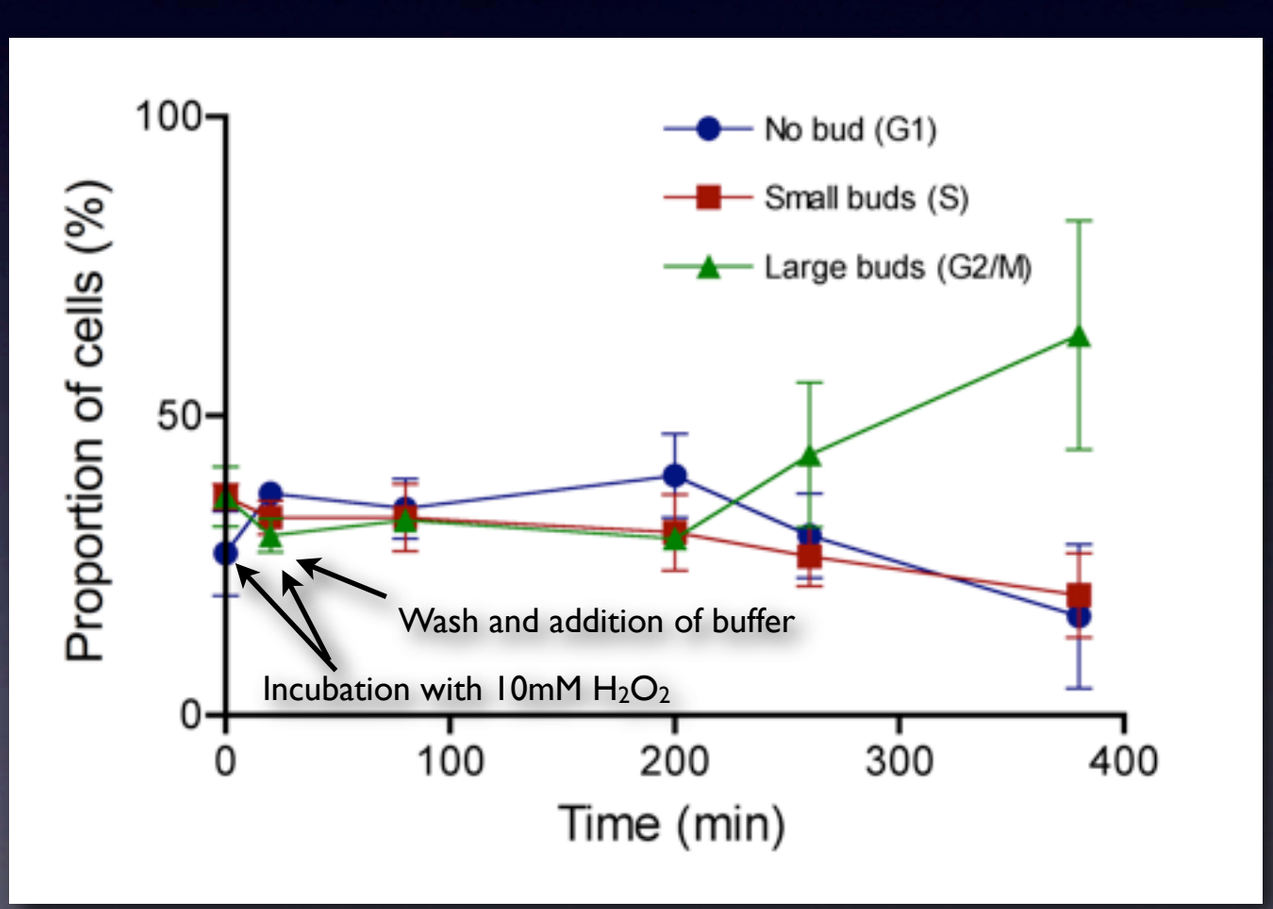


Yeast cell cycle can be assessed by cell morphology

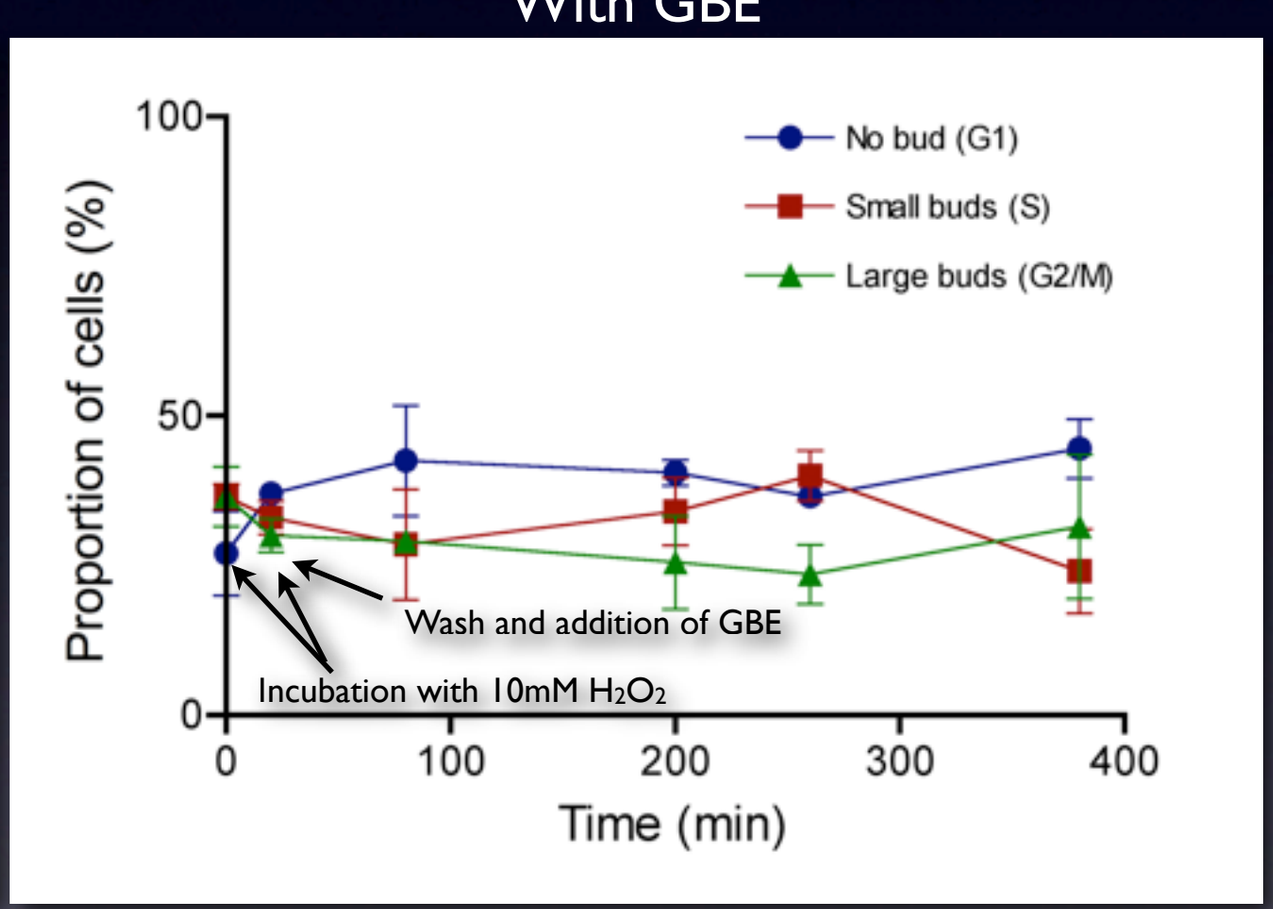




Effect of GBE on cell cycle arrest upon H_2O_2 treatment

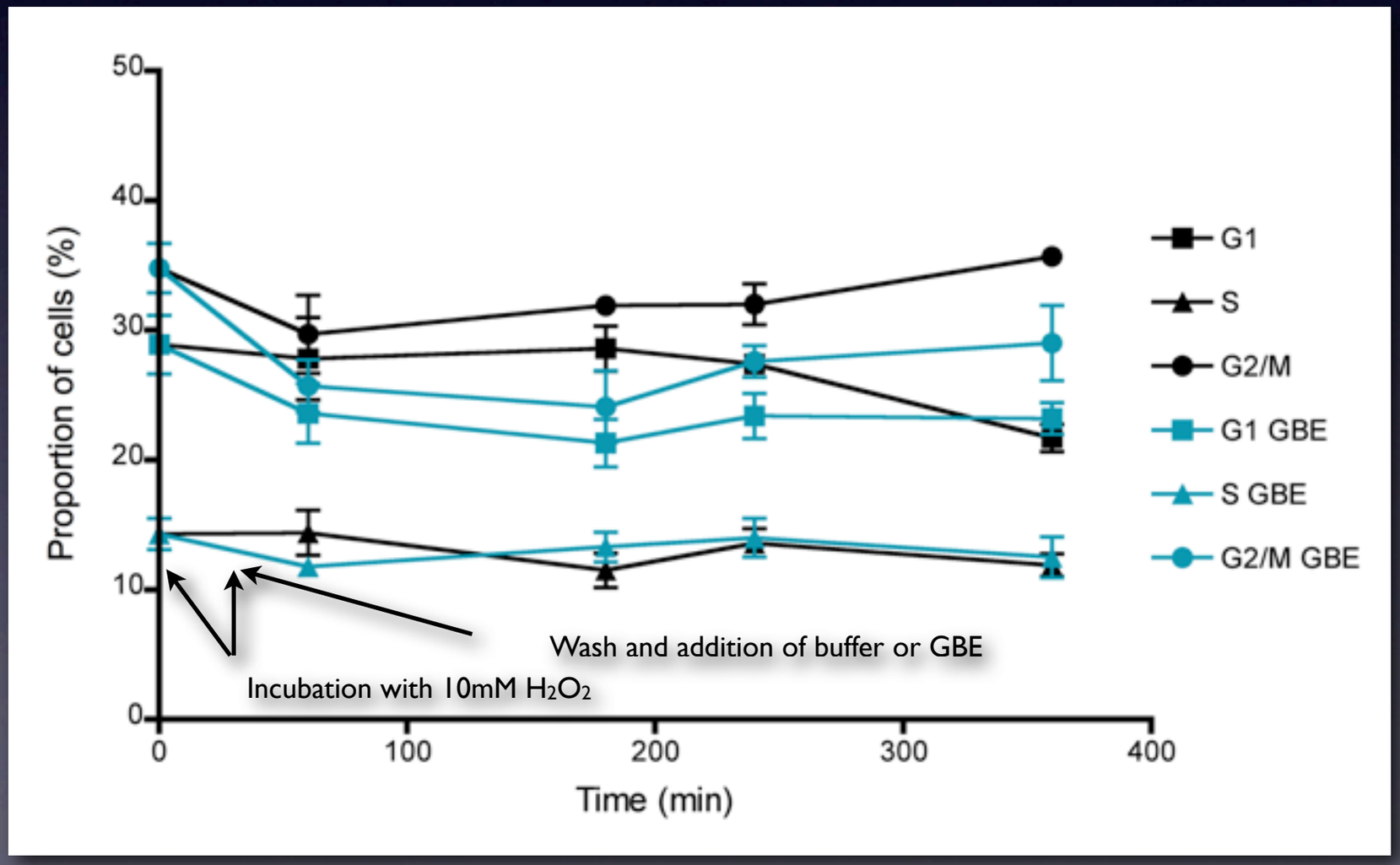


With GBE



Effect of GBE on cell cycle arrest upon H_2O_2 treatment

Cell cycle analysis by flow cytometry with SYBR-Green



Conclusions

- Comet assay is suitable in yeast cells for assessment of DNA damage and DNA damage repair
- GBE improves cell viability under oxidative stress
- GBE protects yeast cells from oxidative DNA damage
- GBE improves DNA damage repair
- GBE decreases intracellular oxidation
- GBE abrogates cell cycle arrest in G2/M upon oxidative shock
- Yeast mutants allow to gain important insights on genotoxicity and antigenotoxicity mechanisms

Acknowledgements

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