Catabolite repression by galactose in overexpressed *GAL4* strains of *Saccharomyces cerevisiae*

T. LODI, C. DONNINI and I. FERRERO*

Institute of Genetics, University of Parma, Viale delle Scienze, 43100 Parma, Italy

(Received 31 December 1990; accepted 13 February 1991)

Catabolite repression by galactose was investigated in several strains of *Saccharomyces cerevisiae* grown on different carbon sources. Galactose repressed as much as glucose; raffinose was less effective. Full derepression was achieved with lactate. The functions tested were L-lactate ferri-cytochrome *c* oxidoreductase, NAD-glutamate dehydrogenase, and respiration. Galactose repression was observed only in the *GAL4* but not in the *gal4* strain. The presence of multiple copies of the *GAL4* gene enhanced the repression by galactose. Different alleles of the *GAL4* gene and the copy number did not affect glucose repression.

Introduction

Several experimental observations on the effect of carbon source on the level of a number of enzymes in *Saccharomyces cerevisiae* have led to the conclusion that glucose severely inhibits the synthesis of both mitochondrial (Slonimski, 1953; Polakis *et al.*, 1965; Duntze *et al.*, 1969; Perlman & Mahler, 1974) and cytoplasmic enzymes (Gancedo *et al.*, 1965; Polakis & Bartley, 1965; Gorts, 1967; Duntze *et al.*, 1969). Growth on oxidizable carbon sources permits a complete expression of those genes repressed by glucose. Among a number of fermentable carbon sources, galactose has been used extensively, and has been considered a nonrepressing (Szekely & Montgomery, 1984) or weakly repressing (Gancedo *et al.*, 1967; Mahler & Lin, 1978) carbon source. However, it has been reported that galactose represses the synthesis of several enzymes to the same extent as glucose (Polakis & Bartley, 1965; Eraso & Gancedo, 1984).

The utilization of galactose depends on a number of enzymes that, together with their regulatory elements, are known as the Gal system. The Gal system is a glucose-repressible catabolic system, inducible by galactose. The inducer may cause the dissociation of GAL80 (a negative regulatory element) from the GAL4 protein (a positive regulatory element) or it may prevent the GAL80 protein from inhibiting the function of the

GAL4 protein, thus permitting expression of the structural genes (for a review see Johnston, 1987).

The GAL4 protein, which is a positive regulatory element in the GAL system, has been shown to be a negative regulatory element in other systems. It has been demonstrated that a multicopy plasmid carrying the *GAL4* gene inhibits the transcription of certain genes lacking the GAL4 binding sites. The negative effect of the GAL4 protein is counterbalanced by high levels of expression of *GAL80* (Gill & Ptashne, 1988).

We present evidence of the repressing effect of galactose on enzymes not directly involved in galactose catabolism. Galactose repression was observed in *GAL4* cells, but not in *gal4* mutants, and depended on the copy number of the *GAL4* gene i.e. repression was more severe in a *gal4* strain transformed with a multicopy plasmid carrying the *GAL4* gene than in a strain carrying the *GAL4* nuclear allele.

Methods

Strains and genetic methods. The yeast strains are listed in Table 1. *Escherichia coli* strain JM83 (*ara*, *rpsL*, Δ (*lac-proAB*), Φ 80; *lacZ*, Δ M15) was used for plasmid amplification. Conjugation, sporulation and tetrad analysis were performed according to Mortimer & Hawthorne (1969) and Sherman *et al.* (1986).

Growth and sporulation media. M medium contained 5 g l⁻¹ Difco yeast extract and 10 g l⁻¹ Difco bacto-peptone supplemented with different carbon sources (glucose 20 g l⁻¹, galactose 20 g l⁻¹, lactate 10 g l⁻¹, raffinose 20 g l⁻¹). Mineral minimal medium (Magni & von Borstel, 1962) was supplemented with 20 g l⁻¹ glucose, and appropriate amino acids, uracil, or adenine at a final concentration of 40 μ g ml⁻¹.

Abbreviations: NAD-GDH, NAD-glutamate dehydrogenase (EC 1.4.1.3); L-LCR, L-Lactate ferri-cytochrome *c* oxidoreductase (EC 1.1.2.3).

Table 1. *Saccharomyces cerevisiae* strains used

Strain	Genotype	Source
D261-9A	α <i>his1 trp1 trp GAL</i>	Parma collection
D273-10B	α prototroph <i>GAL</i>	Obtained from Dr A. Tzagoloff
D234-10B	α <i>trp1 ade1 GAL</i>	Obtained from Dr F. Sherman
W303-1A	α <i>ade2-1 ura3-1 his3-11 his3-15 leu2-3 leu2-112 trp1-1 can1-100 GAL</i>	Gif-sur-Yvette collection
G4- α -5B	α <i>gal4 ade8 MAL CUP^r</i>	Obtained from Dr R. Contopoulou
WG4	α/α diploid prototroph	Cross W303-1A $\times$ G4- α -5B
WG4-6A	α <i>ade his3 GAL</i>	Sporulation of WG4
WG4-6C	α <i>gal4 ura3 ade</i>	Sporulation of WG4

Sporulation medium was VB (Magni & von Borstel, 1962). LB medium was prepared as described in Maniatis *et al.* (1982). For plating, the media were supplemented with 2% Difco Bacto agar.

Preparation of cell free extract. Cells grown to 1×10^8 cells ml⁻¹ were harvested by centrifugation, washed once with distilled water and then washed with 1/25 volume extraction buffer (0.03 M-Tris/HCl, pH 8.5, 1 mM-PMSF). The cell pellet was resuspended in extraction buffer, and cells were disrupted by vortexing at 4 °C in the presence of an equal volume of glass beads (0.5 mm diameter). The suspension was then centrifuged at 5000 g for 5 min at 4 °C. The pellet was discarded, and the supernatant used for enzyme activity measurements.

Enzyme assays. L-lactate ferricytochrome *c* oxidoreductase (EC 1.1.2.3) activity was measured spectrophotometrically at 600 nm, and at a standard temperature of 30 °C in the presence of 50 mM-phosphate buffer (pH 7), 10 mM-sodium azide, 30 μ M-phenazine methosulphate, 50 μ M-2,6-dichlorophenol-indophenol, 30 mM-L-lactate (lithium salt) and an amount of cell-free extract corresponding to approximately 0.1 mg ml⁻¹ in the reaction mixture. The exogenous substrate was added to the reaction mixture after exhaustion of endogenous substrates. NAD-glutamate dehydrogenase (EC 1.4.1.3) activity was assayed as described by Grisolia *et al.* (1964).

Cytochrome spectra. Differential spectra of a suspension of 60 mg wet weight of cells ml⁻¹ were recorded at room temperature in 1 ml 0.1 M-phthalate-KOH, pH 5.0, using a Cary 219 spectrophotometer. The band width was 1 nm and the scan speed was 0.5 nm s⁻¹. The cell suspension reduced by sodium dithionite corresponded to approximately 200 mg wet weight.

Respiration measurements. Cultures grown to 1×10^8 cells ml⁻¹ were harvested by centrifugation, washed twice with cold (4 °C) distilled water and diluted twofold in cold water with respect to pellet volume. The respiratory rate was measured at 30 °C using a Clark electrode in a reaction vessel with 3 ml of air-saturated respiration buffer (0.1 M-phthalate buffer, pH 5.0) containing glucose (10 mM). The reaction was initiated by the addition of 20 mg of cells (wet wt). Respiratory rates were expressed as μ l of O₂ consumed per h per mg of dry mass (Q_{O_2}). Dry wt was determined by weighing 1 ml cell suspension (obtained by mixing each gram of cells (wet wt) with 2 ml of water), and oven-drying overnight at 90 °C. The values used for the determination of respiratory activity are the means from three independent measurements.

Northern analysis. Total RNA isolation and formamide agarose gel electrophoresis were carried out according to Sherman *et al.* (1986). Northern blotting to Hybond N filter (Amersham) and hybridization to ³²P-labelled probe were performed using standard techniques (Maniatis *et al.*, 1982). Probes were labelled with a nick translation kit (Boehringer).

DNA preparation and transformations. Plasmids were prepared by an alkaline lysis protocol (Maniatis *et al.*, 1982). *Escherichia coli* was transformed according to the technique of Mandel & Higa (1970). Yeast transformation was carried out by the LiCl procedure of Ito *et al.* (1983).

Results

Effect of different carbon sources on the level of the enzymes NAD-glutamate dehydrogenase and of L-lactate ferricytochrome c oxidoreductase in various strains

The question of the effect of galactose on the level of enzymes not directly related to its metabolism is still controversial. This carbon source is considered non-repressing by some researchers and repressing by others. Strain-dependent and/or enzyme-dependent sensitivity to galactose may account for this controversy. To investigate this possibility, the effect of galactose on the level of two enzymes was analysed in various strains. The enzymes, NAD-glutamate dehydrogenase (NAD-GDH) and L-lactate ferricytochrome *c* oxidoreductase (L-LCR), were chosen because they are sensitive to glucose catabolite repression (Somlo, 1965; Beck & von Meyenburg, 1968), and NAD-GDH is a cytoplasmic enzyme whereas L-LCR is a mitochondrial enzyme. The strains were chosen because they are widely used in various laboratories.

To analyse the effect of galactose on the level of the two enzymes, the strains were used to inoculate media containing lactate as non-repressing carbon source, either alone or in the presence of 2% (w/v) galactose, glucose or raffinose respectively. The activity of L-LCR in all the strains analysed, grown under non-repressing conditions (lactate), was significantly higher than that obtained in glucose or galactose (Table 2). In addition, a strain-dependent sensitivity to galactose and to glucose was observed. An exception was strain G4- α -5B, which was not repressed by galactose. When the cells were grown on lactate plus raffinose the enzyme activities were never as repressed as those obtained on lactate plus glucose.

Table 2. *Effect of growth in different carbon sources on the level of L-lactate ferricytochrome c oxidoreductase in various strains*

Strain genotypes are listed in Table 1. Enzyme activity was measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of 3 independent experiments. In no case was the variation higher than 15%.

Strain	Carbon sources (w/v)			
	1% lactate	1% lactate + 2% galactose	1% lactate + 2% glucose	1% lactate + 2% raffinose
D261-9A	409	130	159	294
D273-10B	487	118	206	298
D234-10B	319	116	110	304
W303-1A	470	97	89	280
G4- α -5B	328	312	25	302

Table 3. *Effect of growth in different carbon sources on the level of NAD glutamate dehydrogenase*

Strain genotypes are listed in Table 1. Enzyme activity was measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of 3 independent experiments. In no case was the variation higher than 15%.

Strain	Carbon sources (w/v)			
	1% lactate	1% lactate + 2% galactose	1% lactate + 2% glucose	1% lactate + 2% raffinose
D261-9A	306	117	105	245
D273-10B	232	111	127	174
D234-10B	280	121	134	253
W303-1A	375	105	121	240
G4- α -5B	405	417	132	315

With NAD-GDH (Table 3), the degree of repression exerted by galactose was quite similar to that exerted by glucose in all the strains but G4- α -5B. In fact, in this strain the addition of galactose to the culture medium did not determine repression on the enzyme level. The strain was as glucose-repressible as the others. When the cells were grown on lactate plus raffinose, the enzyme activities were never as repressed as with glucose.

Effect of the GAL4 allele on the level of L-lactate ferricytochrome c oxidoreductase and NAD-glutamate dehydrogenase in cultures grown in the presence of galactose

Because the *gal4* strain G4- α -5B did not undergo galactose repression, in order to analyse the role of the *GAL4* gene in galactose repression, the effect of the growth on galactose was measured in strains carrying different alleles, i.e. either *gal4*, monocopy *GAL4* or multicopy *GAL4*.

To obtain strains with a similar genetic background, strain G4- α -5B (*gal4*) was crossed with strain W303-1A carrying the *GAL4* allele. After sporulation of the diploid WG4, a tetrad dissection was done to obtain a *GAL4* strain and a *gal4* strain. The *gal4* character was scored on galactose plus ethidium bromide (20 μ g ml⁻¹) since the growth on galactose of *gal4* strains is impaired in the absence of mitochondrial functions (Douglas & Hawthorne, 1964; Algeri *et al.*, 1981).

In Table 4 the values of L-LCR and NAD-GDH are reported in strains WG4-6A (*GAL4*) and WG4-6C (*gal4*) grown on lactate with and without 2% (w/v) galactose. The addition of galactose to the cultures dramatically lowered the level of the enzymes in the *GAL4* strain. The *gal4* strain was not affected by 'galactose catabolite repression', but was as glucose-repressible as WG4-6A (*GAL4*). To determine whether restoration of galactose repression was associated with a restoration of galactose

Table 4. *Effect of growth in various carbon sources on L-lactate ferricytochrome c oxidoreductase and NAD-glutamate dehydrogenase of strains carrying different alleles of the GAL4 gene*

WG4-6A and WG4-6C are two haploid strains from the same tetrad, obtained by sporulating the diploid strain WG4. WG4-6C/G is the strain WG4-6C transformed by a multicopy plasmid YEP24 containing the regulatory *GAL4* gene. All experimental conditions are as described in Tables 2 and 3.

Strain	L-LCR			NAD-GDH		
	1% lactate	1% lactate + 2% galactose	1% lactate + 2% glucose	1% lactate	1% lactate + 2% galactose	1% lactate + 2% glucose
WG4-6A (<i>GAL4</i>)	394	131	67	405	143	115
WG4-6C (<i>gal4</i>)	407	416	54	387	374	98
WG4-6C/G (<i>GAL4</i>)	415	67	59	398	69	105

Fig. 1. Northern blot analysis of *CYB2* transcripts from (a) WG4-6A (*GAL4*) and (b) WG4-6C (*gal4*) strains. Total cellular mRNA was prepared from cells grown on (1) 1% (w/v) lactate or (2) 1% (w/v) lactate plus 2% (w/v) galactose. Hybridization was carried out using as a probe the nick-translated plasmid pGB5C2H2 (Guiard, 1985), which contains the *CYB2* gene in pBR328. Each lane contained 20 µg of total mRNA.

Table 5. Effect of growth in various carbon sources on respiration of strains carrying different alleles of the *GAL4* gene

WG4-6A and WG4-6C are two haploid strains from the same tetrad, obtained by sporulating the diploid strain WG4. WG4-6C/G is the strain WG4-6C transformed with a multicopy plasmid YEp24 containing the regulatory *GAL4* gene. Oxygen consumption was measured as described in Methods. All values are means of 3 independent experiments. In no case was the variation higher than 10%.

Strain	QO_2 with carbon source indicated (w/v) (µl O_2 h ⁻¹ mg ⁻¹)		
	1% lactate	1% lactate + 2% galactose	1% lactate + 2% glucose
WG4-6A (<i>GAL4</i>)	87	41	9
WG4-6C (<i>gal4</i>)	82	84	8
WG4-6C/G (<i>GAL4</i>)	85	43	10

metabolism, the strain WG4-6C was transformed with the regulatory gene *GAL4*. The *GAL4* gene was carried on the multicopy plasmid YEp24 (obtained from M. Johnston) (Laughon & Gesteland, 1984). Overexpression of the *GAL4* gene rendered strain WG4-6C galactose repressible for both enzymes (Table 4).

Northern analysis of *CYB2* expression in galactose

The L-LCR or cytochrome *b*₂ is a protein synthesized in the cytoplasm and localized in the intermembrane space

Fig. 2. Absorption spectra of (a, b) WG4-6A (*GAL4*), (c, d) WG4-6C (*gal4*) and (e, f) WG4-6C/G (*gal4* transformed with a multicopy plasmid carrying the *GAL4* gene), grown on (a, c, e) 1% lactate or (b, d, f) 1% lactate plus 2% (w/v) galactose.

of mitochondria. Import into the mitochondrion is a multistep process in which several proteins and low-molecular-mass molecules (GTP, ATP) are involved. Moreover, import requires an energized mitochondrial membrane (Gasser *et al.*, 1982; Pfanner *et al.*, 1988). Therefore, cytochrome *b₂* synthesis can be regulated both transcriptionally and post-translationally. To discover at which level 'galactose repression' was exerted, a Northern analysis of L-LCR expression was carried out. RNAs were extracted from the *GAL4* and *gal4* strains grown on media supplemented with lactate and lactate+galactose. After agarose gel electrophoresis and blotting, the filter was hybridized with ³²P-labelled plasmid pGB5C2H2, kindly given to us by B. Guiard (Guiard, 1985), containing the *CYB2* gene in the vector pBR328.

The level of *CYB2* transcript was independent of the carbon sources in the *gal4* strain. On the other hand, the level of transcript in the *GAL4* strain was lowered when galactose was added to the growth medium (Fig. 1).

Effect of the GAL4 gene on the respiratory structures and function of Saccharomyces cerevisiae

To analyse whether the allelic status of the *GAL4* gene was also involved in regulation of respiration, the respiratory activity and absorption spectra of cytochromes were measured in a strain carrying the *gal4* allele, in a strain carrying a single copy of the *GAL4* allele and in a strain carrying multiple copies of the *GAL4* allele, respectively. The results obtained (Table 5) indicated that the dominant allele *GAL4*, either singly or in multicopy, caused a two-fold repression of respiration. The inhibition of respiration in monocopy or multicopy *GAL4* strains when grown on lactate plus galactose was less pronounced than the repression observed when the same strains were grown on lactate plus glucose.

Growth on lactate plus galactose did not affect the cytochrome profile in strains carrying the *gal4* allele, or in the strain carrying a single copy of the *GAL4* allele, but caused a decrease of the peak corresponding to cytochromes *cc₁* (approximately at 550 nm) in the strain carrying the *GAL4* allele in multicopies (Fig. 2). This indicates that 'galactose repression' acts at the biosynthetic level.

Discussion

The data reported here demonstrate that growth on galactose causes repression of the synthesis of the mitochondrial enzyme L-lactate ferricytochrome *c* oxidoreductase and the cytoplasmic enzyme NAD-glutamate dehydrogenase. A less severe repression of respiration was observed. This effect was observed only in

GAL4 cells, and not in *gal4* cells, and was dependent on the copy number of the *GAL4* gene. Appreciable differences were observed between the *GAL4* and *gal4* strains transformed with a multicopy plasmid. A number of hypotheses can be formulated in order to explain the observed effect. In Hypothesis 1, galactose *per se* is a repressing molecule. Its effect is only detectable when it can enter the cell. Sugar entrance is permitted by the positive regulatory effect of the *GAL4* gene product on the inducible permease encoded by *GAL2* gene (Johnston, 1987). In Hypothesis 2, the repressing effect of the galactose is due to a metabolic intermediate produced during its catabolism, therefore it can be observed only in *GAL4* but not in *gal4* cells, since the *gal4* mutants are unable to metabolize it. In Hypothesis 3, the cause of the observed repressed phenotype is the fermentative metabolism of the cells growing on galactose. Finally, in Hypothesis 4, the *GAL4* gene product is responsible for the observed repression. The presence of galactose is necessary since only under these experimental conditions does the *GAL4* gene product become 'available'.

Hypothesis 3 may be refuted, as, if this were the case, the induction of the Gal system by galactose could not occur, since the Gal system is extremely sensitive to catabolite repression. Further, functions such as respiration which are strongly repressed by glucose are poorly repressed by galactose. The possibility that a metabolic intermediate of the catabolism of galactose causes the repression (Hypothesis 2) can be refuted for the reasons above, since the metabolic intermediates of galactose catabolism are the same as those of glucose metabolism, and should repress the Gal system as well. The only metabolites that could negatively affect the enzymes analysed and positively affect the Gal system (induction) are galactose-1-phosphate, glucose-1-phosphate and UDP-galactose. For the same reason, the unmodified galactose molecule could be the effector of the observed phenotype.

Hypothesis 4, that the *GAL4* gene product is the effector, cannot be refuted, since no contradiction exists between the induction of Gal system and repression of other systems. We favour this hypothesis because repression of enzyme synthesis (L-LCR, NAD, GDH, cytochromes) was more severe when the *GAL4* gene was present on a multicopy plasmid. In particular, the peak corresponding to cytochrome *cc₁* which, under our experimental conditions, and in the strains analysed, is poorly sensitive to galactose repression, is strongly lowered only when the *GAL4* gene is present on a multicopy plasmid.

These results suggest that 'catabolite repression' is exerted by galactose, a carbon source which has been and is normally considered a non-repressing carbon source. That the effect exerted by galactose acts at the level of

gene expression is demonstrated by the coincidence of decreasing enzyme activity and mRNA level, at least for lactate dehydrogenase, under all the experimental conditions analysed. The question of what is 'truly' responsible for the observed repression remains to be elucidated, although the *GAL4* copy number does influence the degree of repression, favouring this gene product as a crucial one in the observed repression.

We thank Jack von Borstel for the critical reading of the manuscript. This research was supported by a grant from the Ministero della Pubblica Istruzione (Italy).

References

- ALGERI, A. A., BIANCHI, L., VIOLA, A. M., PUGLISI, P. P. & MARMIROLI, N. (1981). *IMP1/imp1*: a gene involved in the nucleomitochondrial control of galactose fermentation in *Saccharomyces cerevisiae*. *Genetics* **97**, 27–44.
- BECK, C. K. & VON MEYENBURG, H. K. (1968). Enzyme pattern and aerobic growth of *Saccharomyces cerevisiae* under various degrees of glucose limitation. *Journal of Bacteriology* **96**, 479–486.
- DOUGLAS, H. C. & HAWTHORNE, D. C. (1964). Enzymatic expression and genetic linkage controlling galactose utilization in *Saccharomyces*. *Genetics* **49**, 837–844.
- DUNTZE, W., NEUMANN, D., GANCEDO, J. M., ATZPODIEN, W. & HOLZER, H. (1969). Studies on the regulation and localization of the glyoxylate cycle enzymes in *Saccharomyces cerevisiae*. *European Journal of Biochemistry* **10**, 83–89.
- ERASO, P. & GANCEDO, J. M. (1984). Catabolite repression in yeasts is not associated with low levels of cAMP. *European Journal of Biochemistry* **141**, 195–198.
- GANCEDO, C., SALAS, M. L., GINER, A. & SOLS, A. (1965). Reciprocal effects of carbon sources on the levels of AMP-sensitive fructose-1,6-diphosphatase and phosphofructokinase in yeast. *Biochemical and Biophysical Research Communications* **20**, 15–20.
- GANCEDO, C., GANCEDO, J. M. & SOLS, A. (1967). Metabolite repression of fructose 1,6-diphosphatase in yeast. *Biochemical and Biophysical Research Communications* **26**, 518–521.
- GASSER, S. M., OHASHI, A., DAUM, G., BOHNI, P. C., GIBSON, J., REID, G. A., YONETANI, T. & SCHATZ, G. (1982). Imported mitochondrial proteins cytochrome *b₂* and cytochrome *c₁* are processed in two steps. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 267–271.
- GILL, G. & PTASHNE, M. (1988). Negative effect of the transcriptional activator *GAL4*. *Nature, London* **334**, 721–724.
- GORTS, C. P. M. (1967). Effect of different carbon sources on the regulation of carbohydrate metabolism in *Saccharomyces cerevisiae*. *Antonie van Leeuwenhoek* **33**, 451–463.
- GRISOLIA, S., QUISADO, C. L. & FERNANDEZ, M. (1964). Glutamate dehydrogenase from yeast and from animal tissues. *Biochimica et Biophysica Acta* **81**, 61–70.
- GUIARD, B. (1985). Structure, expression and regulation of a nuclear gene encoding a mitochondrial protein: the yeast L(+)-lactate cytochrome *c* oxidoreductase (cytochrome *b₂*). *EMBO Journal* **4**, 3265–3272.
- ITO, H., FUKADA, Y., MURATA, K. & KIMURA, A. (1983). Transformation of intact cells treated with alkali cations. *Journal of Bacteriology* **153**, 163–168.
- JOHNSTON, M. (1987). A model fungal gene regulatory mechanism: the *GAL* genes of *Saccharomyces cerevisiae*. *Microbiological Reviews* **51**, 458–476.
- LAUGHON, A. & GESTELAND, F. R. (1984). Primary structure of the *Saccharomyces cerevisiae* *GAL4* gene. *Molecular and Cellular Biology* **4**, 260–267.
- MAGNI, G. & VON BORSTEL, R. C. (1962). Different rates of spontaneous mutation during mitosis and meiosis in yeast. *Genetics* **47**, 1097–1108.
- MAHLER, H. R. & LIN, C. C. (1978). Molecular events during the release of α -aminolevulinate dehydratase from catabolite repression. *Journal of Bacteriology* **135**, 54–61.
- MANDEL, H. & HIGA. (1970). Calcium dependent bacteriophage DNA infection. *Journal of Molecular Biology* **53**, 159–162.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular cloning: a laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- MORTIMER, R. K. & HAWTHORNE, D. C. (1969). Yeast genetics. In *The Yeasts*, vol. 1. Edited by A. H. Rose & J. S. Harrison. New York: Academic Press.
- PERLMAN, P. & MAHLER, H. R. (1974). Derepression of mitochondria and their enzymes in yeast: Regulatory aspects. *Archives of Biochemistry and Biophysics* **162**, 248–271.
- PFANNER, N., HARTL, F. U. & NEUPERT, W. (1988). Import of proteins into mitochondria: a multi-step process. *European Journal of Biochemistry* **175**, 205–212.
- POLAKIS, E. S. & BARTLEY, W. (1965). Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. *Biochemical Journal* **97**, 284–297.
- POLAKIS, E. S., BARTLEY, W. & MEEK, G. A. (1965). Changes in the activities of respiratory enzymes during the aerobic growth on different carbon sources. *Biochemical Journal* **97**, 298–304.
- SHERMAN, F., FINK, G. R. & HICKS, J. B. (1986). *Laboratory Course Manual for Methods in Yeast Genetics*. pp. 143–149. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- SLONIMSKI, P. P. (1953). *La Formation des Enzymes Respiratoires chez la Levure*. Paris: Masson et Cie Editeurs.
- SOMLO, M. (1965). Induction des lactico-cytochrome *c* reductases (D- et L-) de la levure aerobie par des lactates (D- et L-). *Biochimica et Biophysica Acta* **97**, 183–201.
- SZEKELY, E. & MONTGOMERY, D. L. (1984). Glucose represses transcription of *Saccharomyces cerevisiae* nuclear genes that encode mitochondrial components. *Molecular and Cellular Biology* **4**, 939–946.