YEAST VOL. 11: 25–32 (1995)

An Efficient Method to Isolate Yeast Genes Causing Overexpression-mediated Growth Arrest

CARME ESPINET, MARIA ANGELES DE LA TORRE, MARTI ALDEA AND ENRIQUE HERRERO*

Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, Rovira Roure 44, 25198-Lleida, Spain

Received 17 June 1994; accepted 10 September 1994

In order to characterize new yeast genes regulating cell proliferation, a number of overexpression-sensitive clones have been isolated from a *Saccharomyces cerevisiae* cDNA library in a multicopy vector under the control of the *GAL1* promoter, on the basis of growth arrest phenotype under galactose-induction conditions. Thirteen of the independent clones isolated in this way correspond to previously known genes (predominantly coding for morphogenesis-related proteins or for multifunctional transcriptional factors), while the remaining 11 independent clones represent new genes with unknown functions. The more stringent conditions employed in this screening compared with previous ones that also employed a dominant genetics approach to isolate overexpression-sensitive genes has allowed us to extend the number of yeast genes that exhibit this phenotype. The effect of overexpression of *MCM1* (whose product participates in the regulation of a number of apparently unrelated cellular functions) has been studied in more detail. Galactose-induced overexpression of *MCM1* leads to rapid growth arrest at the G_1 or S cell cycle stages, with many morphologically-abnormal cells. Several of the other clones also exhibit a G_1 arrest terminal phenotype when overexpressed.

KEY WORDS — *Saccharomyces cerevisiae*; dominant genetics; growth regulation; *MCM1*.

INTRODUCTION

Dominant genetics has been used for the identification of *Saccharomyces cerevisiae* genes whose overexpression causes special phenotypes (Liu *et al.*, 1992; Ramer *et al.*, 1992). Genomic (Ramer *et al.*, 1992) and cDNA (Liu *et al.*, 1992) galactose-inducible expression libraries in *GAL1* promoter-based centromeric vectors lend limited numbers of positive clones in screenings for overexpression-associated lethality. Genes involved in cell cycle regulation or in the modulation of other physiological events whose products participate in multiprotein regulatory complexes may be finely regulated themselves in order to attain precise levels of the protein. It is characteristic of many of these regulatory complexes to share some of their components with other multimers (Forsburg and Nurse, 1991). Thus, it seems plausible that overexpression of some of the proteins may titrate out other components of functionally different complexes, therefore affecting separate cellular functions and leading to growth inhibition. With this rationale dominant genetics may be employed to isolate genes involved in growth regulation whose overexpression leads to proliferation arrest. The use of dominant genetics for this purpose would be more productive if more extreme overexpression conditions than those of previous studies were employed. Here we report the construction of a *S. cerevisiae* cDNA library in an episomal vector under the *GAL1* promoter, and the isolation and partial characterization of a large number of clones that show growth arrest in galactose-containing growth media.

MATERIALS AND METHODS

Library construction

The episomal plasmid pYES2 (Invitrogen) was employed as vector. It contains *URA3* as selective marker and the *GAL1* promoter followed by a multiple cloning site region. To construct the library, *S. cerevisiae* OL1 cells (Gil *et al.*, 1991) were grown exponentially in YEPD medium at

*Corresponding author.

CCC 0749–503X/95/010025–08

30°C, and polyadenylated RNA was obtained using the Quick Prep mRNA Purification kit from Pharmacia. cDNA was synthesized with a Riboclone cDNA Synthesis kit (Promega) by using the *Not*I primer adaptor. Blunt-ended cDNA fragments were digested with *Not*I and cloned into alkaline phosphatase-treated pYES2 DNA digested downstream of the *GAL1* promoter with *Eco*lCRI (*Sac*I) and *Not*I. Ligation mixtures were electroporated into *Escherichia coli* MC1061 (Ausubel *et al.*, 1989). The library thus obtained contains about $1{\cdot}5 \times 10^6$ independent recombinant clones.

Isolation of overexpression-sensitive library clones

Library DNA was transformed (Gallego *et al.*, 1993) into *S. cerevisiae* INVSc2 (*MAT*α *his3 ura3 GAL2*) (Invitrogen) and 10 000 transformants were screened for absence of growth in galactose-based SD minimal medium (Rose *et al.*, 1990) plates at 30°C. From 44 transformants that grew in glucose but not in galactose medium, plasmid DNA was isolated (Rose *et al.*, 1990) and retransformed into *S. cerevisiae* CML72 (*MAT***a** *GAL2 his3 leu2 ura3 trp1*). In all cases, galactose-dependent growth arrest was confirmed.

Sequencing

For each of the overexpression-sensitive clones, more than 200 bp were sequenced by the dideoxy method (Sanger *et al.*, 1977) from the T7 primer in the pYES2 vector into the adjacent cDNA (which corresponds to the 5′ region of the cloned gene), using a T7 DNA Sequencing kit (Pharmacia). DNA sequence analysis was carried out with the GCG software package, and Genebank and EMBL databases were used for homology searching.

Growth conditions

SD minimal medium (Rose *et al.*, 1990) supplemented with 2% carbon source (usually glucose) and the required amino acids was employed. For induction experiments, 2% galactose was added to cultures that contained 2% raffinose as carbon source. YEPD (1% yeast extract, 2% peptone, 2% glucose) was used as rich medium. Cells were grown at 30°C.

Other methods

General genetic methods were carried out as described in Rose *et al.* (1990). DAPI staining and other microscopy procedures were done as described in Pringle *et al.* (1989). Budding index was determined by observing at least 100 formaldehyde-fixed cells for each sample under a phase-contrast microscope. DNA content distributions were obtained by flow cytometry as described by Tyers and Futcher (1993) with an Epics Profile (Coulter Co.) cytofluorimeter. Alpha-factor production was studied by the halo method (Sprague, 1991).

RESULTS

Isolation of overexpression-sensitive clones from a GAL1 *promoter-based expression library in a multicopy vector*

We constructed a yeast cDNA library in pYES2 in order to isolate clones that show growth arrest in galactose-induced overexpression conditions. The isolated clones corresponded to 24 different cDNAs, represented from one to four times (Table 1). Thirteen of these cDNAs showed complete homology with previously identified *S. cerevisiae* genes. They included genes coding for cytoskeletal proteins (*ACT1*), proteins involved in vesicular transport and permeability (*SEC17, ERG6, ARF2*), regulators of mating type and pheromone-inducible genes (*MCM1, SIR1, ATE1*), putative components of signal transduction pathways (*RHO1*), stress resistance-related products (*HSF1, NTH1*), a common subunit for RNA polymerases I and III (*SRP40*), an activator of the general amino acid permease Gap1 (*AUA1*) and the non-histone protein A (*NHP6A*). *ACT1* as well as *NHP6B* (coding for isoform B of the non-histone protein A), had been isolated in previous screenings (Liu *et al.*, 1992; Ramer *et al.*, 1992) using a related approach. The other 11 different cDNAs did not show significant DNA homologies with genes from either yeast or other organisms. These are named *SHE1* to *11* (for sensitivity to high expression) (Table 1).

Arrest phenotype in the overexpression conditions

Arrest phenotype was not uniform among the different transformants. While no growth (compared with controls) was observed after 5 days in streaks on SD-galactose plates, when galactose was added to cells grown in SD-raffinose liquid medium and growth was monitored for 8 h, the rate at which cells arrested proliferation was diverse (Table 1). Overexpression of some of the

Table 1. Isolated cDNAs causing growth arrest when overexpressed.

Gene or cDNA*	Number of isolates	Gene product or function	Growth pattern in SD-galactose medium†	Relative budding index in over-expression conditions‡
ACT1	4	Actin 1	−	0·63
ARF2	2	ADP-ribosylation factor 2	−	1·19
ATE1	1	Arginyl tRNA protein transferase	±	0·68
AUA1	3	Amino acid uptake activator	−	0·32
ERG6	1	S-adenosylmethionine: C24 sterol methyltransferase	−	0·93
HSF1	1	Heat-shock transcription factor	−	0·35
MCM1	1	Transcription factor for mating type-specific genes	+	0·07
NHP6A	4	Non-histone protein 6A	−	0·56
NTH1	4	Neutral trehalase	−	0·99
RHO1	4	Ras-related GTP-binding protein	+	0·27
SEC17	1	Endoplasmic reticulum membrane-protein involved in secretion	−	0·81
SIR1	1	Silent mating type information regulator	−	1·20
SRP40	2	RNA polymerases I and III common subunit	±	0·84
SHE1	1		±	0·01
SHE2	2		−	0·88
SHE3	3		−	1·04
SHE4	2		−	0·53
SHE5	1		ND	ND
SHE6	1		−	0·70
SHE7	1		±	0·79
SHE8	1		±	0·40
SHE9	1		+	0·75
SHE10	1		−	0·62
SHE11	1		−	0·44

*EMBL accession numbers for new cDNAs are as follows: *SHE1*: X78018; *SHE2*: X78019; *SHE3*: X78020; *SHE4*: X78021; *SHE5*: X78022; *SHE6*: X78023; *SHE7*: X78024; *SHE8*: X78025; *SHE9*: X78026; *SHE10*: X78027; *SHE11*: X78028.
†CML72 transformants with plasmids carrying the respective cDNAs in the pYES2 vector were grown exponentially in SD-raffinose liquid medium at 30°C. Galactose (2%, final concentration) was added and evolution of optical density (at 600 nm) was followed during the next 8 h as a measure of cell growth. − : rapid growth arrest after 3–4 h from galactose addition. ± : slow growth rate (<70% relative to control cells transformed with pYES2) in SD-raffinose plus galactose. +: no differences in growth rate relative to control cells during the initial 8 h in SD-raffinose plus galactose.
‡Percentages of budded cells were quantified microscopically after 8 h in SD-raffinose plus galactose. Values are relative to those of control cultures of cells transformed with pYES2, which are given the unit value.
ND: Not determined.

cDNAs (i.e. *ERG6, HSF1, MCM1, RHO1, SHE1, SHE11*) caused most of the cells to become unbudded, which is characteristic of arrest at the G_1 stage of the cell division cycle. Cells transformed with *MCM1, RHO1* or *SHE1* were considerably larger than control cells after 8 h of overexpression (data not shown), a fact that may explain why increase of optical density is not significantly affected in the period studied, while cell division is inhibited. In contrast, in other cases (such as *ERG6, HSF1* or *SHE11*), G_1 arrest was not accompanied by cell enlargement.

The overexpression effect can be modulated by the presence of glucose in the growth medium

We have found that expression under the control of the *GAL1* promoter can be modulated by the addition of low concentrations of glucose. We tested the dependence of the level of *GAL1*-directed expression of *MCM1, SHE1* and *RCS1* [a cell cycle regulator whose overexpression also leads to growth arrest (Gil *et al.*, 1991, and our unpublished results)] on cell growth. For the three constructions, the respective transformants were

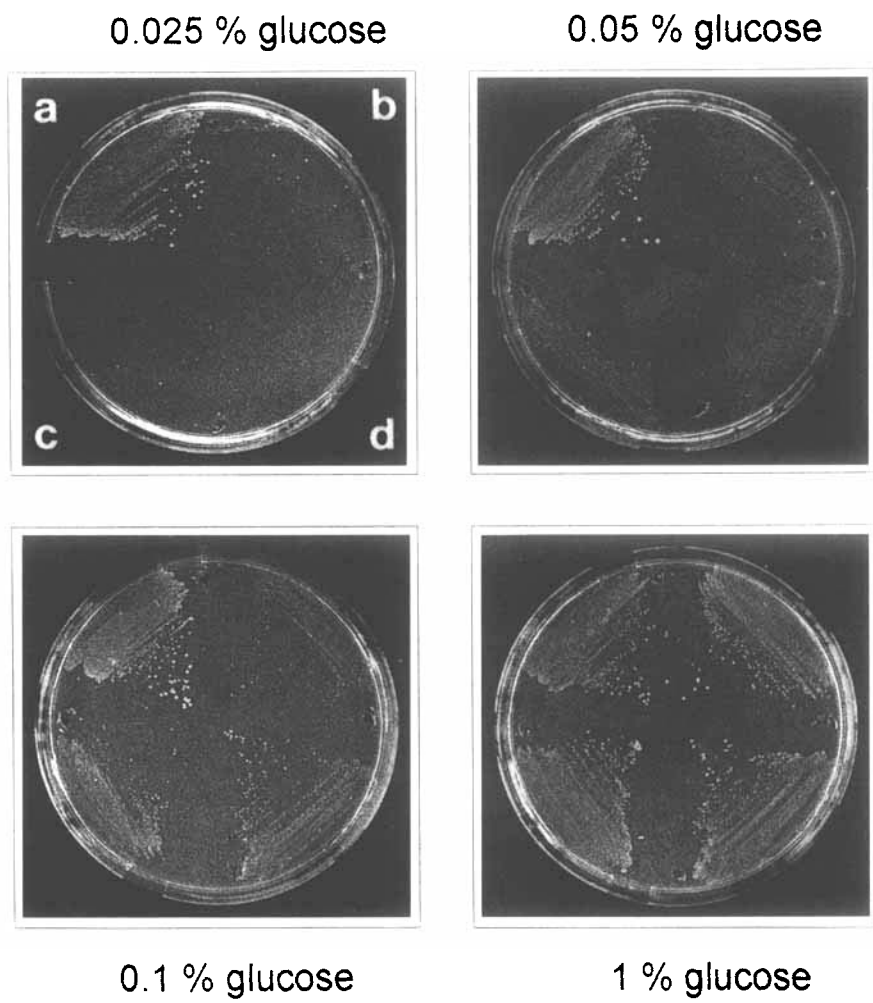

Figure 1. Effect of glucose concentration on growth of *S. cerevisiae* CML72 transformants overexpressing *RCS1* (b), *MCM1* (c) or *SHE1* (d) under the *GAL1* promoter control. Cells transformed with the pYES2 vector (sections a) or with the respective plasmids were plated on SD agar containing 2% galactose plus the indicated glucose concentration, and plates were incubated for 72 h at 30°C.

able to grow (albeit at decreased growth rate than the transformants with the vector alone) in SD-raffinose plus galactose medium also containing 0·05 or 0·1% glucose concentration (Figure 1). At higher glucose concentrations growth was indistinguishable from control cultures lacking galactose.

Effect of MCM1 *overexpression on cell growth and morphology*

The *S. cerevisiae MCM1* product is an essential multifunctional protein (Kirkman-Correia *et al.*, 1993; Lydall *et al.*, 1991; Messenguy and Dubois, 1993; Passmore *et al.*, 1989). We have studied more extensively the phenotype of *MCM1*-overexpressing cells. In these conditions cell division was inhibited after 2 h in galactose medium (Figure 2A), in contrast with control cells not overexpressing *MCM1*. Concomitantly, most of the cells appeared unbudded, and after 8 h of galactose induction practically no budded cells were observed (Figure 2B), which is consistent with arrest at the G_1 stage of the cell division cycle. FACS analysis (Figure 2C) confirmed that most of the cells were in fact at G_1 (1N DNA content),

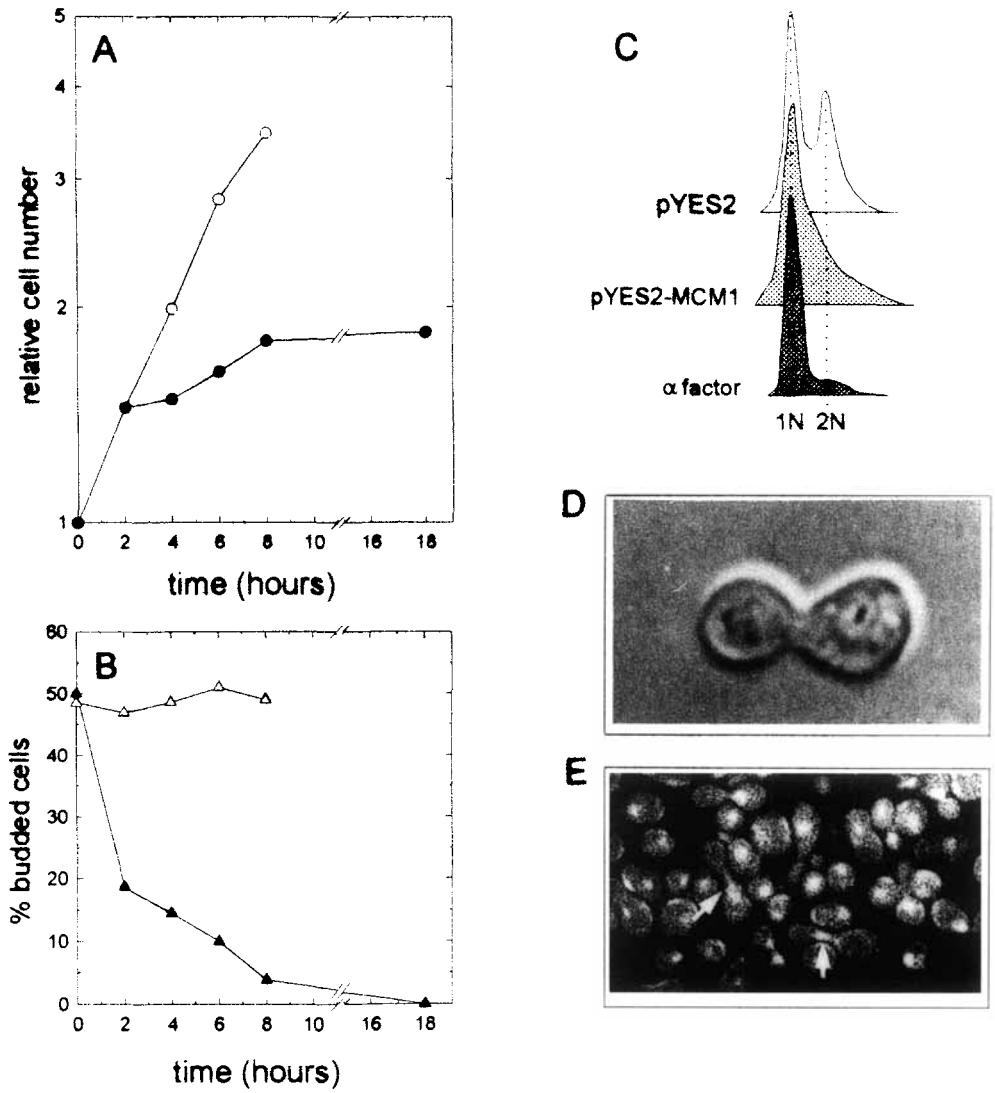

Figure 2. Effect of *MCM1* overexpression on cell growth. *S. cerevisiae* CML72 cells transformed with the pYES2 vector or with a pYES2-based plasmid carrying the *GAL1-MCM1* construction were grown in SD-raffinose medium at 30°C. At time 0, galactose at 2% concentration was added, and incubation was continued for the indicated times. (A) Relative cell number per ml in cultures of cells transformed with pYES2 (○) or with plasmid containing the *GAL1-MCM1* construction (●). (B) Percentage of budded cells at different times after galactose addition in cultures of pYES2 transformants (△) or of cells transformed with the *GAL1-MCM1* plasmid (▲). (C) DNA analysis by flow cytometry of cells transformed with pYES2 or with the pYES2-based *GAL1-MCM1* plasmid, after 8 h of galactose addition; as a control, the results obtained with CML72 cells after 90 min of treatment with α-factor (1 μg/ml) are also shown. (D) Micrograph of a cell overexpressing *MCM1* after 8 h in the presence of galactose (magnification 2500 ×). (E) DAPI staining of cells overexpressing *MCM1*; arrows show elongated cells with a nucleus at the centre of the cell (magnification 1000 ×).

although a shoulder of cells probably at S phase appeared; such a shoulder was not observed in α factor-treated control cells, which are known to become synchronously arrested at the Start point of the G_1 stage. *MCM1*-overexpressing cells exhibited abnormal morphologies, with significant

numbers of elongated and pear-shaped cells (Figure 2D). The percentage of abnormally-shaped cells was maximum (about 20%) after 4 and 6 h in galactose medium, and decreased at later times (data not shown). About 2% of the cells had a zygote-like structure with a single nucleus at the centre of the cell as revealed by DAPI staining (Figure 2E). Since Mcm1p is involved in regulation of mating type-specific genes (Passmore *et al.*, 1989; Kirkman-Correia *et al.*, 1993), the possibility arose that its overproduction in **a**-type cells could cause synthesis of α-factor by the latter and consequently the ability of these **a**-cells to mate with cells of the same mating type. To test it, CML72 transformants carrying a plasmid with the *GAL1-MCM1* construction were patched over a lawn of tester **a**-cells (K2112 strain, *MAT***a** *ho-βgal ura3 ade2-1? can1-100 met his3 leu2-3 leu2-112 trp1-1 bar1::HisG URA3*, obtained from K. Nasmyth) on SD medium plus 0·05% glucose, 2% galactose and the auxotrophic requirements. No halo of growth inhibition of the tester cells due to α-factor production by the CML72 transformants could be detected (data not shown). Also, when the CML72 cells transformed with the *GAL1-MCM1* plasmid were mixed with CML67 cells (*MAT***a** *leu2 ura3 ade2 trp1*) in SD medium containing 0·05% glucose and 2% galactose to induce Mcm1p overproduction, and then plated on selective glucose minimal medium to allow growth of diploid cells exclusively, no such diploid colonies arose (data not shown).

DISCUSSION

Growth arrest caused by overexpression of the genes reported here may be due to different reasons. Expression level of cytoskeletal proteins such as actin 1 is critical for normal growth (Liu *et al.*, 1992; and the results reported here). The *RHO1* protein is essential for cell growth and has been shown to be a GTP-binding activator of the *CDC43* geranylgeranyltransferase (Qadota *et al.*, 1992), being itself a modulator of actin cytoskeleton in the budding process (Adams *et al.*, 1990). The *NHSP6A* product (and that of its homologous *NHSP6B*) functions downstream of a MAP kinase pathway regulating yeast cell polarity and morphogenesis (Costigan *et al.*, 1994). In a broad sense, Sec17p could also be considered as a morphogenetic protein since as a yeast homologue of the mammalian transport factor α-SNAP, it is involved in vesicular transport between the endoplasmic reticulum and the Golgi apparatus (Griff *et al.*, 1992).

Another group of genes isolated in this work could be included in the category of stress-related genes. Overexpression of *HSF1* under the *GAL1* promoter in a centromeric vector has been shown to increase the transcription level of the heat-shock protein hsp70, and to slightly decrease the growth rate in a wild-type strain (Sorger and Pelham, 1988). *AUA1* in high copy number vectors causes hypersensitivity of the cell to several amino acids and related compounds that are toxic (Sophianopoulou and Diallinas, 1993). The *NTH1* product catalyses the degradation of trehalose, which is involved in protection of the cell from stress conditions such as nutrient starvation (Werner-Washburne *et al.*, 1993). However, the exact physiological role of these stress-related genes is unclear, so that it is difficult to explain the relationship between their overexpression and growth arrest.

The approach described in this work was initially designed to isolate genes coding for multifunctional regulatory proteins. The isolation of *MCM1* illustrates the validity of the approach. In fact, the *MCM1* product is a DNA binding protein (Kuo and Grayhack, 1994) that participates in transcription regulatory complexes with apparently different cellular functions, such as expression of mating-type-specific genes (Passmore *et al.*, 1988; Kirkman-Correia *et al.*, 1993), mating-type switching (Lydall *et al.*, 1991) or arginine metabolism (Messenguy and Dubois, 1993). *MCM1*-overexpressing cells arrested at the G_1 or S stages of the cell cycle. The fact that a fraction of the cells become arrested at the S phase may be related to the proposed additional role of the *MCM1* product as a DNA replication factor (Maine *et al.*, 1984; Christ and Tye, 1991). Since the whole population of cells overexpressing *MCM1* remained unbudded, it can be hypothesized that in these conditions spurious initiations of DNA replication might occur without passing the Start point of the cell cycle, which would be deleterious for the cells.

Another gene included in the same category of transcription regulators would be *SIR1*, which represses transcription of the silent mating-type genes as part of a multiprotein complex (Chien *et al.*, 1993; Rine and Herskowitz, 1987; Stone *et al.*, 1991). Overexpression of *SIR1* in multicopy vectors titrates out other factors required for repression of gene expression at telomers (Chien

et al., 1993); higher levels of *GAL1*-driven *SIR1* expression attained in the conditions employed in this work could be detrimental for the cell by abolishing the telomeric silencing effect, which would lead to unregulated expression of telomere genes.

The isolation of the *MCM1* clone in this study points to the advantage of using strong induction conditions for an exhaustive search of clones showing overexpression-sensitive growth. *MCM1* has also been isolated employing the dominant genetics approach as a dominant activator of the mating response in the absence of sexual pheromone (Ramer *et al.*, 1992). However, in that study overexpression did not cause growth arrest, perhaps due to employing a centromeric vector and to the long distance between the *GAL1* promoter and the *MCM1* initiation codon in the isolated clone. Other genes such as *AUA1* (Sophianopoulou and Diallinas, 1993), *HSF1* (Sorger and Pelham, 1988), *SEC17* (Griff *et al.*, 1992), *SIR1* (Chien *et al.*, 1993) and *RHO1* (Qadota *et al.*, 1992) have also been introduced in yeast cells in multicopy vectors without causing the growth arrest effects described here. The number of overexpression-sensitive genes isolated in the present study is larger than the number of genes with similar phenotype isolated in previous studies (Liu *et al.*, 1992; Ramer *et al.*, 1992). This is probably due to the fact that we have employed more stringent conditions, that is *GAL1*-based induction for genes cloned in a multicopy vector.

Overexpression of *PMA1* or *PMA2* genes, coding for two closely related plasma membrane ATP-ases of *S. cerevisiae*, has toxic effects on cells, causing growth delay and accumulation of intracellular membranous structures (Eraso *et al.*, 1987; Supply *et al.*, 1993). It might be expected that overproduction of integral membrane proteins would cause deleterious effects on cell ultrastructure due to disruption of normal membrane compartmentalization. Among the previously characterized genes that we have isolated in the present study, only one of them, *SEC17*, is known to be membrane-associated. This would be far below the number of expected genes considering that a significant fraction of yeast genes may code for integral membrane proteins (Goffeau *et al.*, 1993), although the possibility remains that some of the not yet characterized *SHE* genes may also encode integral membrane proteins. That our method does not seem to enrich for membrane protein genes may be explained by the fact that the corresponding mRNAs could constitute a minor subpopulation in the culture conditions employed for library construction, or alternatively by the fact that overproduction of membrane proteins, although toxic for the cells in some cases, might not lead to complete growth arrest. It must also be kept in mind that post-translational modifications required for correct membrane targeting may not necessarily occur when proteins are overproduced.

By using *GAL1*-driven expression partially repressed by low glucose concentrations (i.e. 0·05–0·1%), the cDNA library in pYES2 should also be useful to isolate new genes by other screening methods not based on growth arrest or lethality.

ACKNOWLEDGEMENTS

We thank X. Gómez for introducing us to cytofluorimetry techniques and X. Calomarde for his technical assistance in microscopy. This work was supported by grant PB91-0237 of DGICYT, with additional financial aid from the Ajuntament de Lleida.

REFERENCES

Adams, A. E. M., Johnson, D. I., Longnecker, R. M., Sloat, B. F. and Pringle, J. R. (1990). *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell. Biol.* **111,** 131–142.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1989). *Current Protocols in Molecular Biology*. Wiley, New York.

Chien, C.-T., Buck, S., Sternglanz, R. and Shore, D. (1993). Targeting of SIR1 protein establishes transcriptional silencing at *HM* loci and telomeres in yeast. *Cell* **75,** 531–541.

Christ, C. and Tye, B. K. (1991). Functional domains of the yeast transcription/replication factor *MCM1*. *Genes Dev.* **5,** 751–763.

Costigan, C., Kolodrubetz, D. and Snyder, M. (1994). *NHP6A* and *NHP6B*, which encode HMG1-like proteins, are candidates for downstream components of the yeast *SLT2* mitogen-activated protein kinase pathway. *Mol. Cell. Biol.* **14,** 2391–2403.

Eraso, P., Cid, A. and Serrano, R. (1987). Tight control of the amount of yeast plasma membrane ATPase during changes in growth conditions and gene dosage. *FEBS Lett.* **224,** 193–197.

Forsburg, S. L. and Nurse, P. (1991). Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and

Schizosaccharomyces pombe. Annu. Rev. Cell Biol. **7,** 227–256.

Gallego, G., Casas, C. and Herrero, E. (1993). Increased transformation levels in intact cells of *Saccharomyces cerevisiae* aculeacin A-resistant mutants. *Yeast* **9,** 523–526.

Gil, R., Zueco, J., Sentandreu, R. and Herrero, E. (1991). *RCS1*, a gene involved in controlling cell size in *Saccharomyces cerevisiae. Yeast* **7,** 1–14.

Goffeau, A., Nakai, K., Slominski, P. and Risler, J. L. (1993). The membrane proteins encoded by yeast chromosome III genes. *FEBS Lett.* **325,** 112–117.

Griff, I. C., Schekman, R., Rothman, J. E. and Kaiser, C. A. (1992). The yeast *SEC17* gene product is functionally equivalent to mammalian α-SNAP protein. *J. Biol. Chem.* **267,** 12106–12115.

Kirkman-Correia, C., Stroke, I. L. and Fields, S. (1993). Functional domains of the yeast STE12 protein, a pheromone-responsive transcriptional activator. *Mol. Cell. Biol.* **13,** 3765–3772.

Kuo, M. H. and Grayhack, E. (1994). A library of yeast genomic MCM1 binding sites contains genes involved in cell cycle control, cell wall and membrane structure, and metabolism. *Mol. Cell. Biol.* **14,** 348–359.

Liu, H., Krizek, J. and Bretscher, A. (1992). Construction of a *GAL1*-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast. *Genetics* **132,** 665–673.

Lydall, D., Ammerer, G. and Nasmyth, K. (1991). A new role for MCM1 in yeast: cell cycle regulation of *SW15* transcription. *Genes Dev.* **5,** 2405–2419.

Maine, G. T., Sinha, P. and Tye, B. K. (1984). Mutants of *S. cerevisiae* defective in the maintenance of minichromosomes. *Genetics* **106,** 365–385.

Messenguy, F. and Dubois, E. (1993). Genetic evidence for a role for MCM1 in the regulation of arginine metabolism in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **13,** 2586–2592.

Passmore, S., Elble, R. and Tye, B. K. (1989). A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.* **3,** 921–935.

Pringle, J. R., Preston, R. A., Adams, A. E. M., Stearns, T., Drubin, D. G., Haarer, B. K. and Jones, E. W. (1989). Fluorescence microscopy methods for yeast. *Meth. Cell Biol.* **31,** 357–435.

Qadota, H., Ishii, I., Fujiyama, A., Ohya, Y. and Anraku, Y. (1992). *RHO* gene products, putative small GTP-binding proteins, are important for activation of the *CAL1/CDC43* gene product, a protein geranylgeranyltransferase in *Saccharomyces cerevisiae. Yeast* **8,** 735–741.

Ramer, S. W., Elledge, S. J. and Davis, R. W. (1992). Dominant genetics using a yeast genomic library under the control of a strong inducible promoter. *Proc. Natl. Acad. Sci. USA* **89,** 11589–11593.

Rine, J. and Herskowitz, I. (1987). Four genes responsible for a position effect on expression from *HML* and *HMR* in *Saccharomyces cerevisiae. Genetics* **116,** 9–22.

Rose, M. D., Winston, F. and Hieter, P. (1990). *Methods in Yeast Genetics: A Laboratory Course Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, F. G., Nicklen, S. and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.

Sophianopoulou, V. and Diallinas, G. (1993). *AUA1*, a gene involved in ammonia regulation of amino acid transport in *Saccharomyces cerevisiae. Mol. Microbiol.* **8,** 167–178.

Sorger, P. K. and Pelham, H. R. B. (1988). Yeast heat-shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell* **54,** 855–864.

Sprague, G. F. Jr. (1991). Assay of yeast mating reaction. *Meth. Enzymol.* **194,** 77–93.

Stone, E. M., Swanson, M. J., Romeo, A. M., Hicks, J. B. and Sternglanz, R. (1991). The *SIR1* gene of *Saccharomyces cerevisiae* and its role as an extragenic suppressor of several mating-defective mutants. *Mol. Cell. Biol.* **11,** 2253–2262.

Supply, P., Wach, A., Thinès-Sempoux, D. and Goffeau, A. (1993). Proliferation of intracellular structures upon overexpression of the PMA2 ATPase in *Saccharomyces cerevisiae. J. Biol. Chem.* **268,** 19744–19752.

Tyers, M. and Futcher, B. (1993). Far1 and Fus3 link the mating pheromone signal transduction pathway to three G_1-phase Cdc28 kinase complexes. *Mol. Cell. Biol.* **13,** 5659–5669.

Werner-Washburne, M., Braun, E., Johnston, G. C. and Singer, R. A. (1993). Stationary phase in the yeast *Saccharomyces cerevisiae. Microbiol. Rev.* **57,** 383–401.