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Genome-Wide analysis of yeast transcription upon calcium shortage

L. J. Lombardía,[1] M. Becerra,[1] E. Rodríguez-Belmonte,[1] N. C. Hauser,[2] M. E. Cerdán[1]

[1]Dpto. Biología Celular y Molecular, Universidad de La Coruña, F. Ciencias, Campus de La Zapateira s/n 15075, La Coruña, Spain
[2]Molecular-Genetic Genome Analysis Group, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Summary Several regulatory circuits related to important functions, like membrane excitation, immunoresponse, replication, control of the cell cycle and differentiation, among others, cause an increase in intracellular calcium level that finally has a consequence upon transcription of specific genes. The sequencing of the whole genome of eukaryotic cells enables genome-wide analysis of gene expression under many conditions not yet assessed by conventional methods. Using the array technology, the effect of calcium shortage in yeast cells was studied. Correspondence analysis of data showed that there is a response in transcription that is correlated to calcium shortage. The distribution of up-regulated-genes in functional categories suggests a regulatory connection between the cell-cycle progression and the energetic metabolic requirements for growth and division. In silico analysis of promoters reveals the frequent appearance of the Mlu I cell cycle box (MCB) *cis* element that binds the transcriptional regulatory factor Mcm1.

INTRODUCTION

Calcium signalling mechanisms are used by differenced eukaryotic cells in a wide range of regulated processes as diverse as neuronal excitation [1], activation of T cells during immunoresponse [2], regulation of replicative enzymes [3,4] and other nuclear functions like regulation of nuclear kinases which control cell cycle, transcription mediated by Pol III and pre-mRNA splicing, as well as ribosomal assembly and protein translation [5].

Calmodulin is the major calcium-binding protein in most eukaryotic cells and Ca^{2+} signals can regulate gene expression through the calmodulin-dependent protein phosphatase known as calcineurin and through calmodulin-dependent protein kinases. In the yeast *Saccharomyces cerevisiae* functional homologues of calmodulin [6], calcineurin [7] and calmodulin-dependent protein kinases have been described [8]. There are several physiological conditions, which generate Ca^{2+} signals in yeast. The first one is the long-term stimulus caused by mating pheromones. After pheromone signalling, there is an increase in intracellular Ca^{2+} levels that activate calcineurin and calmodulin-dependent kinases [9,10]. The second physiological condition to be cited is the regulation of cation homeostasis, caused by high salt or high Ca^{2+} in the medium, which is very complex and includes the stimulation of P-type ion pumps [11], Ca-pumping ATPases in the vacuole, Pmc1 [12], or the Golgi complex, Pmr1 [13], as well as inhibition of a vacuolar H^+/Ca^{2+} exchanger Vcx1/Hum1 [14]. The Ca^{2+} signals commented earlier cause gene regulation through the specific transcriptional factor Tcn1/Crz1 [15] that is only targeted to the nucleus after dephosphorylation regulated by calcineurin [16].

The regulatory circuits mentioned earlier are all related to signals that cause an increase in intracellular calcium levels but there are no data available about the effect of calcium shortage. The sequencing of the whole genome of the yeast eukaryotic cell enables genome-wide analysis of gene expression and anticipates to similar and near-coming studies carried out in human cells. In this report we describe a genome-wide survey of changes in gene expression in *S. cerevisiae* cells grown under three conditions with different Ca^{2+} levels.

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Correspondence to: Professor M. Esperanza Cerdán, Dpto. de Biología Celular y Molecular, F. Ciencias, Universidad de A Coruña Campus de A Zapateira s/n, A Coruña 15071, Spain. Tel.: +34-9-81-167000; fax: +34-9-81-167065; e-mail: n.hauser@dkfz-heidelberg.de, bmanamrt@udc.es

MATERIALS AND METHODS

Strains and media

The haploid strain used in this study was FY73 (*Matα ura3-52 his3-200*) from which is derived the diploid strain FY1679 used during the yeast sequencing project [17]. The cells were grown in three different conditions until 0.8 A_{600}. The first one (+Ca) corresponds to a control culture in complete medium, containing 6.7 g/l of Yeast Nitrogen Base (YNB) without amino acids, 4 g/l of dextrose as carbon source and 20 mg/l of uracil and histidine to supplement strain's auxotrophies; the concentration of Ca^{2+} in this medium is 12 mM. YNB was prepared according to the following recipe per liter of culture: 1 g KPO_4H_2, 0.5 g $MgSO_4$, 0.1 g NaCl, 0.1 g $CaCl_2$, 5 g $(NH_4)_2SO_4$, 2 μg biotin, 400 μg calcium pantothenate, 2 μg folic acid, 2 mg inositol, 400 μg niacin, 200 μg *p*-aminobenzoic acid, 400 μg pyridoxine, 200 μg riboflavin, 400 μg thiamine hydrochloride, 500 μg boric acid, 40 μg copper sulphate, 100 μg potassium iodide, 200 μg ferric chloride, 400 μg manganese sulphate, 200 μg sodium molybdate and 400 μg zinc sulphate. The second condition (−Ca) was the same but, omitting $CaCl_2$ and replacing calcium pantothenate by sodium pantothenate in YNB. In the third condition (⇑Ca), half of the cells from condition 2 were submitted to a 45-min calcium pulse by the addition of $CaCl_2$ at a final concentration of 50 mM in the culture medium.

RNA isolations

Cells were harvested and immediately frozen in liquid nitrogen to later on disrupting them with a Micro-Dismembrator (B. Braun Biotech International). The resulting powder was mixed with TRIZOL Reagent (Life Technologies) and total RNA was extracted by the method of Chomczynski and Sacchi [18].

Probe generation

Probe generation was as described previously [19]. Briefly, 60 μg of total RNA was annealed to oligo dT15 and used as a template to synthesise and radiolabel the corresponding first strand cDNA with 50 μCi of [α-^{33}P]dCTP and SuperScript II (Life Technologies). The reactions were carried out at 43 °C for 1 h, after which the RNA was hydrolysed with NaOH at 65 °C for 30 min. The probe was purified by isopropanol precipitation and the isotope incorporation was measured to check the efficiency of the reaction.

Filters hybridisations, washings and stripping

Filters were pre-hybridised during 1 h at 65 °C in the hybridisation mix: 5× SSC, 5× Denhardt's solution and 0.5% SDS. The probe was then denatured for 5 min at 100 °C, cooled quickly on ice and hybridised with the arrays over night at 65 °C. The day after, two washes were carried out at hybridisation temperature for 5 and 20 min, respectively, in 2× SSC, 0.1% SDS. Filter regeneration was done by pouring a boiling solution of 5 mM sodium phosphate (pH 7.5) and 0.1% SDS over the filters prior to their reuse.

Signal quantification

The filters were exposed 24 h to a storage phosphor screen and the data were collected using a PhosphorImager Scanning Instrument 425 (Molecular Dynamics). Signal quantification was performed with Array Vision software (Molecular Dynamics) that localises over each array element a bounding circle fitted to the size of the DNA spot. Local area background was defined placing manually 10 bounding circles throughout the filter. For each condition, the data from four independent hybridisations were analysed, using two different arrays and RNA samples (a total of 8 replica-spots per gene).

Statistical analysis

Taking into account the numerous intrinsic variations of transcriptional profiling on arrays, a stringent statistical analysis [20] was performed to ensure the significance of the conclusions extracted from the data. Signal intensities of repeated hybridisations were normalised and significance levels assessed by two stringency criteria. The highly stringent "min–max separation" is calculated by taking the minimum distance between all data points of the two strains. The less stringent criteria, called "standard deviation separation", is defined as the difference of the means of the two data sets diminished by one standard deviation. In the Tables 1 and 2 (access at http://www.dkfz.de/tbi), a colour code indicates the two stringency measures. According to these criteria, data were classified as being of high, medium or low statistical significance.

Clustering by correspondence analysis (CA)

CA is an explorative computational method for the study of associations between variables. In the analysis of array-based transcript analysis [21], the display of genes and experiments proved very valuable for biological data interpretation. A brief description of the method is summarised below, but reference to the original paper [21] is highly recommended.

CA was originally developed for contingency tables and is intimately connected with the χ^2 test for homogeneity in a contingency table. Much like principle component analysis, it displays a low dimensional projection of the data into a plane (the biplot). The aim is to embed both rows (genes) and columns (hybridisations) of a matrix in the same space. Like other projection methods, CA

Table 1 Genes down-regulated at low calcium levels

ORF	Gene	Function	*I* (+Ca)	*I* (−Ca)	*I* (Ca shift)	*R*	QM
YMR108W	*ILV2*	Isoleucine–valine biosynthesis	159155	45175	66298	−3.5	H
YDR210W		Unknown	46962	16660	18302	−2.8	H
YPR010C	*RPA135*	Transcription	30907	12241	11793	−2.5	H
YDR046C	*BAP3*	Transport	42445	17402	9454	−2.4	H
YLR467W	*YRF1-5*	Unknown	116911	50247	39674	−2.3	H
YDR209C		Unknown	22250	9534	9415	−2.3	H
YBR016W		Unknown	129105	57721	74617	−2.2	H
YNL322C	*KRE1*	Cell wall biogenesis	32185	14339	19862	−2.2	M
YJR109C	*CPA2*	Glutamate metabolism	239336	106758	66207	−2.2	H
YLR399C	*BDF1*	Meiosis	51890	23542	25364	−2.2	M
YOR303W	*CPA1*	Arginine biosynthesis	27891	12746	17721	−2.2	H
YFR028C	*CDC14*	Mitosis	30834	14258	14947	−2.2	M
YGR086C		Unknown	50907	23755	22860	−2.1	M
YFL067W		Unknown	18986	8983	9090	−2.1	M
YGL008C	*PMA1*	H^+ homeostasis	72860	34452	27369	−2.1	M
YOR280C		Unknown	10589	5027	6161	−2.1	M
YKL080W	*VMA5*	Vacuolar acidification	31232	14832	15026	−2.1	M
YGR098C	*ESP1*	Cytoskeleton	53590	25487	29671	−2.1	M
YBL111C		Unknown	88187	42611	38290	−2.1	H
YPR202W		Unknown	26318	12745	13475	−2.1	H
YJR048W	*CYC1*	Oxidative phosphorylation	39275	19105	15846	−2.1	M
YJL179W	*PFD1*	protein folding	68993	33562	29726	−2.1	H
YBL110C			20820	10135	9267	−2.1	H
YDR527W		Unknown	31714	15574	18280	−2.0	M
YEL075C		Unknown	17019	8384	8555	−2.0	M
YGR175C	*ERG1*	Sterol metabolism	28321	13946	15113	−2.0	M
YER028C		Unknown	24906	12354	9561	−2.0	H
YMR018W		Unknown	27897	13907	15005	−2.0	M
YGR119C	NUP57	Nuclear protein targeting	11333	5681	6252	−2.0	M

$R = -$(Control/low calcium); QM: quality measure, H: high-quality regulation, M: mid-quality regulation; *I*: mean of normalised intensities.

Table 2 Physical and functional distribution of up-regulated genes during calcium shortage

Chromosome	Size (kb)	ORF	Gene	Function	*R*	QM
I	230	YAL041W	*CDC24*	Cell growth and division	1.8	M
II	813	YBL005W	*PDR3*	Transcription	1.8	M
		YBR208C	*DUR1,2*	Nitrogen metabolism	2.4	H
		YBR234C	*ARC40*	Cytoskeleton	2.5	M
III	316	YCL020W		GAG protein TyA	2.7	H
		YCL033C*		Unknown	1.9	M
		YCL034W*		Unknown	1.8	H
		YCL035C*	*GRX1*	Oxidative stress response	1.8	H
IV	1532	YDR077W	*SED1*	Unknown	1.8	H
		YDR079W	*PET100*	Respiration	2.5	M
		YDR133C		Unknown	1.9	H
		YDR446W	*ECM11*	Unknown	2.2	M
		YDR472W	*TRS31*	Targeting complex TRAPP	2.1	M
		YDR542		Unknown	1.8	M
V	577	YEL021W	*URA3*	Nitrogen metabolism	2.1	M
		YER034W	*HYP2*	Unknown	1.9	M
VI	270	YFL034W		Unknown	2.1	H
VII	1091	YGL139W		Unknown	1.8	H
		YGR264C	*MES1*	Protein synthesis	1.8	H
VIII	563	YHR003C*		Unknown	2	H
		YHR004C*	*NEM1*	Nuclear organisation	1.8	H
		YHR005C*	*GPA1*	Signal transduction	1.9	H
IX	440	YIL060W*		Unknown	2.9	M
		YIL061C*	*SNP1*	mRNA splicing	2.9	M
		YIL151C		Unknown	2.7	H
X	745	YJL098W*	*SAP185*	Cell growth and division	2.4	H
		YJL099W*	*CHS6*	Chitin biosynthesis	2	H
		YJL145W*		Unknown	2	H
		YJL146W*	*IDS2*	Meiosis	1.9	H
		YJL147C*		Unknown	1.9	H

Table 2 (*Continued*)

Chromosome	Size (kb)	ORF	Gene	Function	*R*	QM
		YJL157C*	*FAR1*	Cell growth and division	2.6	H
		YJL158C*	*CIS3*	Cell wall	3	H
		YJL159W*	*HSP150*	Cell wall	2.8	H
		YJL162C		Unknown	2.5	M
		YJL168C	*SET2*	Transcription	1.9	M
		YJL194W	*CDC6*	Cell growth and division	3.9	M
		YJL217W		Unknown	2.2	H
		YJR016C	*ILV3*	Nitrogen metabolism	1.9	H
		YJR027W*		Unknown	2.1	H
		YJR028W*		GAG protein TyB	2.7	H
		YJR039W″*		Unknown	1.9	H
		YJR041C*		Unknown	1.8	H
		YJR051W	*OSM1*	Fumarate reductase	1.9	M
		YJR063W	*RPA12*	Transcription	1.8	M
		YJR111C*		Unknown	2	H
		YJR112W*	*NNF1*	Cell growth and division	2.8	H
		YJR113C*		Protein biosynthesis	2	H
		YJR122W*	*CAF17*	Transcription	1.9	M
		YJR124C*		Unknown	3.1	H
		YJR126C*		Unknown	2	H
		YJR128W		Unknown	2	M
		YJR138W	*IML1*	Unknown	1.9	H
XI	666	YKL034W		Unknown	2	M
		YKL046C*		Unknown	2.3	H
		YKL047W*		Unknown	2.5	H
		YKL048C*	*ELM1*	Cell growth and division	1.8	H
		YKL057C*	*NUP120*	Transcription	2.1	M
		YKL058W*	*TOA2*	Transcription	2.3	H
		YKL059C*		Unknown	1.9	H
		YKL060C*	*FBA1*	Carbohydrate metabolism	1.8	H
		YKL070W*		Unknown	1.8	M
		YKL072W*	*STB6*	Transcription	2	H
		YKL153W		Unknown	1.8	H
XII	1078	YLR404W		Unknown	2.1	H
XIII	924	YML002W		Unknown	2.3	H
		YML040W		GAG protein TyA	1.9	H
		YML045W		GAG protein TyB	2.5	M
		YML089C		Unknown	2.7	H
		YML095C	*RAD10*	DNA repair	2.4	H
		YML100W*	*TSL1*	Carbohydrate metabolism	2.7	H
		YML101C*		Unknown	2.5	M
		YMR046C*		GAG protein TyA	2.7	H
		YMR048W*		Unknown	2.3	H
		YMR051C*		GAG protein TyA	2.3	H
		YNR057C		Unknown	2.9	H
		YMR070W	*MOT3*	Mating	2	H
		YMR081C	*ISF1*	RNA splicing	1.8	M
XIV	784	YNL094W		Unknown	1.8	M
XV	1091	YOR035C	*SHE4*	Unknown	2.4	M
		YOR212W	*STE4*	Pheromone pathway	1.8	M
XVI	948	YPR124W	*CTR1*	Copper transport	2.5	H

R: activation factor; QM: quality measure; H: high; M: medium.

represents variables such as transcription intensities of genes as vectors in a high-dimensional space; the dimensionality of the space is the number of hybridisations performed. On the other hand, hybridisations can be considered in a gene-dimensional space. Similar objects are clustered together resulting in small distances. In CA, points are depicted such that the sum of the distances of the points to their centroid is proportional to the value of the χ^2 statistics of the data table. Distances among points (among genes or among hybridisations) are not meant to approximate Euclidean distances but rather the χ^2 distance. In contrast, the distance between a gene and a hybridisation cannot be directly interpreted. Lines in the biplot denote the direction of the hybridisations and genes that show strong expression in a certain condition are located in this direction. The farther away from the centroid (intersection of lines), the more pronounced is the association of the genes with this condition. Genes

that are down-regulated in this condition appear on the opposite site of the centroid.

Promoter analysis

In silico analysis of promoters was carried out by the RSA tools facilities (embnet.cifn.unam. mx/~jvanheld/rsa-tools/) in a region expanding up to −1000 bp from the ATG of the genes considered.

RESULTS AND DISCUSSION

CA on the effects of calcium levels on the yeast transcriptome

We have analysed the expression of the yeast genome under three different conditions characterised by differences in calcium availability. The control is a synthetic media usually employed in laboratory cultures (12 mM Ca^{2+}), the second condition is characterised by calcium shortage (1 mM Ca^{2+}), and the third condition is caused by a shift induced over this latest condition adding Ca^{2+} to a final concentration of 50 mM. The transcriptome of *S. cerevisiae* was screened using the high-density membrane hybridisation method [19]. RNA was extracted from the different cultures and labelled cDNAs obtained from each RNA sample were used to probe the DNA arrays containing the whole set of genes [19].

Repeated hybridisations were performed resulting in at least eight data sets each. Data were analysed by statistical procedures, relative changes were calculated and a measure of significance was determined for each individual data point [20], the latter indicated by a colour code in the relevant tables. The complete list for all genes as well as the median of normalised signal intensities can be downloaded from the EUROFAN web page at MIPS (http://mips.gsf.de/proj/eurofan/eurofan_2/b2/index.html).

For a global interpretation of the data, the variations were clustered using a bipole algorithm called CA recently applied to the analysis of microarray data [21]. One big advantage of this procedure is the fact that both the experiments (hybridisations in each given condition) and the genes are clustered and presented in the same biplot. Fig. 1 shows the result of such a clustering applied to the conditions studied in this work. Hybridisations related to the three different conditions analysed are represented by defined coloured squares (see Fig. 1), the median of hybridisation repeats by coloured circles, and expression of individual genes by black dots. The biplot displayed in Fig. 1 shows three directions corresponding to the three conditions analysed; the coloured lines clearly differentiates the direction of the hybridisations. This fact reveals the existence of statistical differences in the specific transcriptional response obtained in each condition. Genes that show strong expression in a certain condition are located in the direction of the corresponding line and there is a direct correlation between the magnitude of the response and the distance from the centroid (intersection of lines). The conclusion from this analysis is that there is a response in transcription that is correlated to calcium availability.

Transcription during calcium shortage

Changes observed in the transcriptome at (−Ca) are caused by increased expression of a set of genes while the number of genes expressed at lower levels is no significant. Indeed, only three genes diminish their expression over 2.5-fold, *YDR210W*, *YMR108W*(*YLV2*) and *YPR010C*(*RPA135*) and this repression is not reversed by the shift to high calcium levels (⇑Ca) (Table 1).

Genes that are expressed at higher levels during calcium shortage, with regulation ratios higher than 1.8 and with high or media quality regulation (as described in the Statistical analysis Section) are included in Fig. 2. A functional classification of these regulated genes reveals that many of them are yet of unknown function and the others can be clustered in six categories: cell wall, carbohydrate metabolism, nitrogen metabolism, cell rescue, cell division, transcription and ionic homeostasis (Table 2). These genes respond to the 45-min calcium shift (Fig. 2) by diminishing their transcription and this behaviour suggests that changes in calcium levels have a regulatory role during a short-term response.

Among the regulated genes related to cell division we found *CDC24*, *FAR1*, *GPA1*, *SAP185*, *CDC6*, *ELM1* and *NNF1*. Cdc24, the guanine-nucleotide exchange factor for the yeast GTPase Cdc42, is a calcium-binding protein that interacts with Far1 and causes cytoskeletal rearrangements during cell cycle and in response to mating pheromones [22]. Stimulus caused by mating pheromones (which increase intracellular Ca^{2+} levels) is mediated in *S. cerevisiae* by a receptor-coupled heterotrimeric G-protein; the beta-gamma subunit of the G-protein stimulates a PAK/MAP kinase cascade that leads to cellular changes during mating. However, the pheromone-responsive G-alfa protein, Gpa1, antagonises the G-beta-gamma-induced signal [23]. The observed induction of the *GPA1* gene during calcium shortage, the opposite signal to high Ca^{2+} generated by mating induction, could have a role in this antagonistic effect upon pheromone signalling (Fig. 2 and Table 2). Cdc6 is a cell division control protein that interacts functionally with ORC, participates in a pre-replication complex and inhibits Cdc28 histone H1 kinase. It has been proved that the level of *CDC6* transcripts increases in late G1, reaching a peak (approximately 10–20-fold over the initial level) at about the G1/S phase boundary [24].

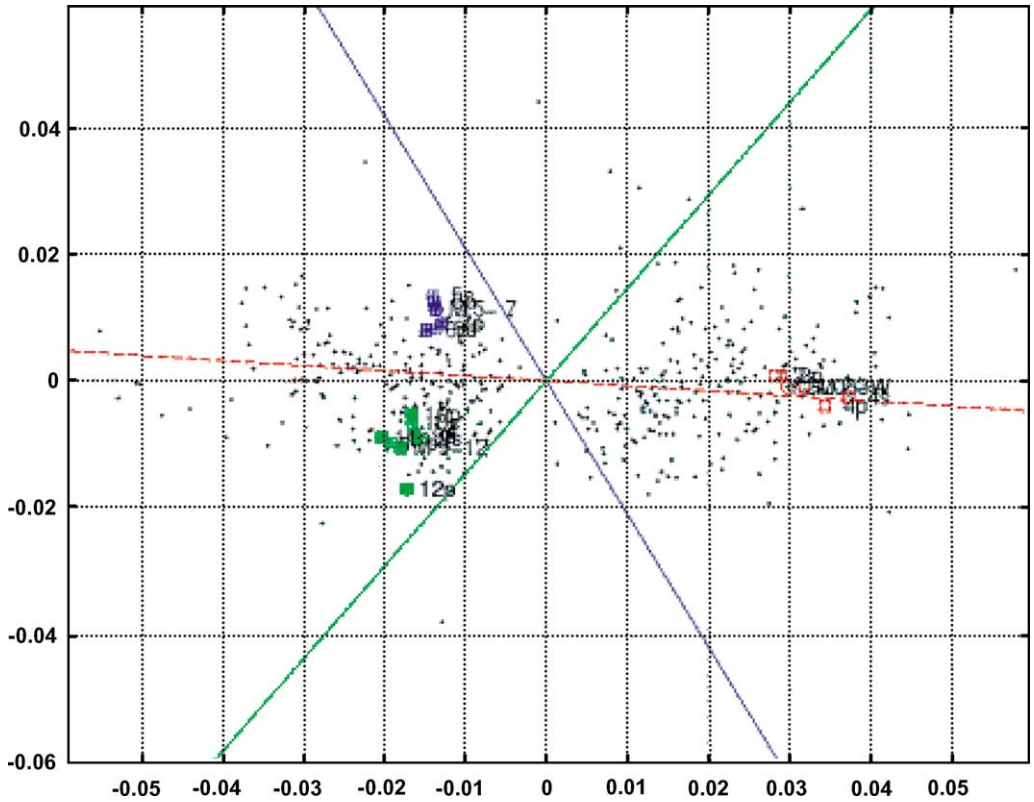

Fig. 1 Biplot obtained by CA of the normalised data from the *Saccharomyces cerevisiae* transcriptome in three conditions characterised by different calcium availability. The coloured squares represent the respective experiment (hybridisation) each repeated four times and each showing the primary (p) and secondary (s) spots for the two repetitions of DNA probes present in these arrays; (+Ca) red (Experiments 1–4), (−Ca) blue (Experiments 5–8), (⇑Ca) green (Experiments 9–12). The coloured circles represent the hybridisation median. The black dots represent intensity signals for the individual genes. The majority of genes, equally transcribed in the three conditions and projecting over the centroid, have been removed to clear the figure. The biplot shows three directions corresponding to the three conditions analysed. Additional explanation is given in MATERIALS AND METHODS Section.

Besides the genes related to cell growth and division, a remarkable number of genes related to carbohydrate and nitrogen metabolism are included in Table 2. This particular distribution in functional categories led us to the hypothesis that calcium levels could act as a multiple purpose signal connecting the cell-cycle progression to the energetic metabolic requirements for growth and division and prompted us to find common regulatory *cis* elements in the promoters of these genes. Several signals, which increase the intracellular calcium levels, cause gene regulation through the specific transcriptional factor Tcn1/Crz1 [15], but at low calcium levels Tcn1 remains in the cytoplasm [16] and, therefore, it cannot function as a transcriptional activator in the nucleus. Analysis of the promoters of genes from Table 2 with the "DNA pattern" facility of RSA tools looking for the two consensus sequences recognised by Tcn1 gives a low proportion of coincidences, even allowing substitutions (only 30% of the promoters have matches and with one mismatch). This is other point of evidence against the participation of this regulatory factor in the up-regulation observed at (−Ca).

The *CDC6* promoter has an Mlu I cell cycle box (MCB) *cis* element defined by the consensus SACGCGNS (S = G or C) that binds Mcm1 [25,26]. When we analysed the promoters of genes, which expression is increased at (−Ca) from Table 2, looking for this MCB pattern and allowing just a single substitution, we found the consensus sequence in 85.1% of these genes. The homology between Mcm1 and the mammalian serum response factor (SRF), which binds DNA after CKII phosphorylation and that, has been involved in cell differentiation and cell proliferation [27–29] makes this finding attractive.

Data from Kuo and Grayhack [30] also showed a connection between Mcm1-binding sites and genes involved in cell-cycle control, cell wall and membrane structure and metabolism. These authors isolated a library of yeast genomic sequences to which Mcm1 is bound, and then identified these targeted genes. Functional groups, and even particular genes like *FAR1* or *HSP150*, detected by their approach are rather coincident to the genes activated at (−Ca) in our array strategy. We may speculate that Mcm1, in a calcium-dependent way, co-ordinates decisions about

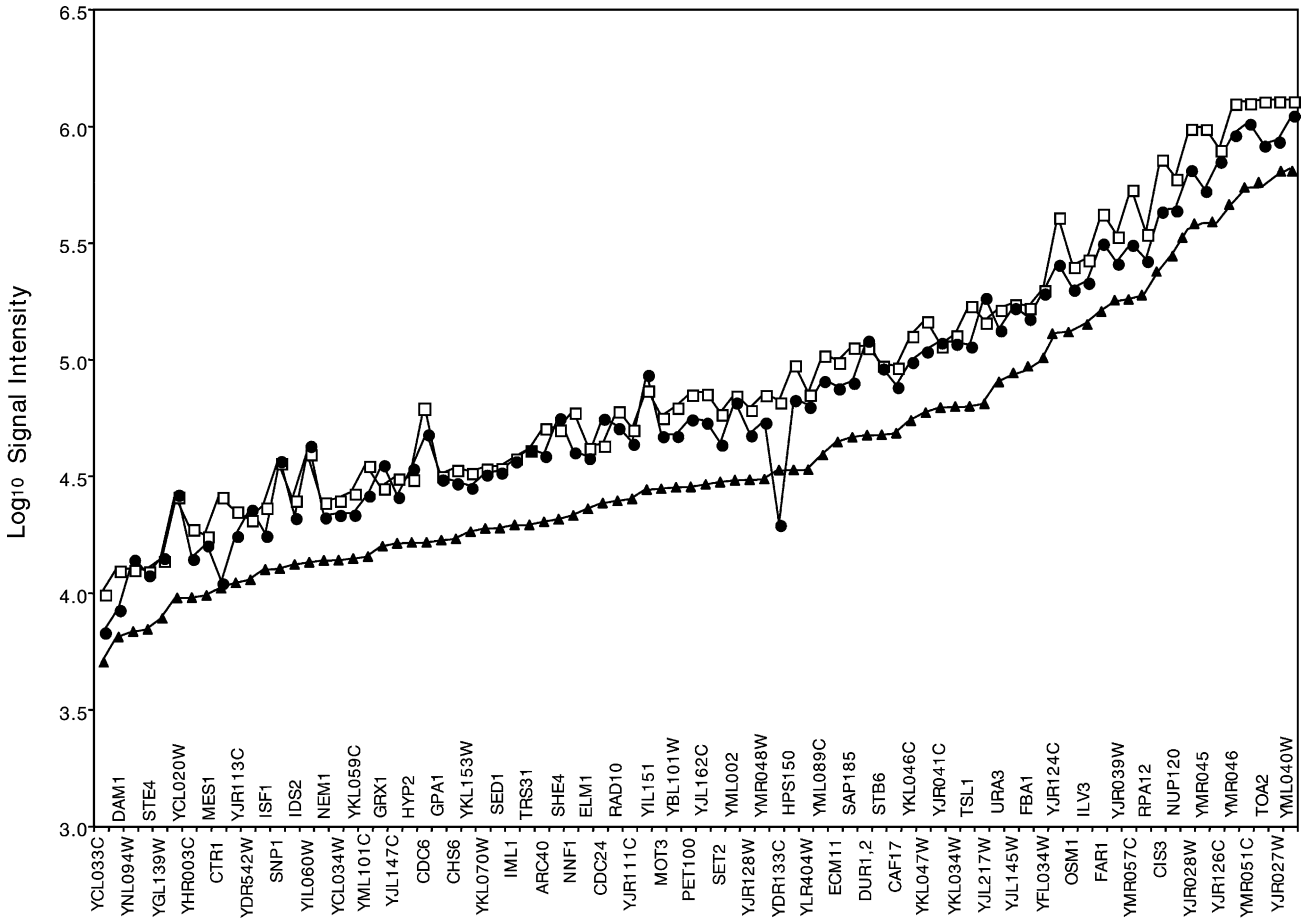

Fig. 2 Genes induced at calcium shortage and change in transcription after calcium shift. +Ca (▲), −Ca (□), ⇑Ca (●). Signal intensity corresponds to the means of normalised values (for statistical significance of each value, http://mips.gsf.de/proj/eurofan/eurofan2/b2/index.html).

cell-cycle progression with changes in cell wall integrity and metabolic activity. Confirmation of this hypothesis will require further experiments to find the nexus between the Mcm1 regulatory protein and calcium signals as well as detailed analysis of Mcm1 binding to the selected promoters. Meanwhile, other point of evidence favours the connection between cell cycle progression and metabolic regulation. Recently the group of Coria [31] has cloned in the yeast *Kluyveromyces lactis* a second G-alfa subunit, *Kl*Gpa2, which is involved in regulating cyclic AMP levels upon glucose stimulation, suggesting a cross-talk between the pathway mediated by *Kl*Gpa1 (related to pheromone signalling and cell division) and glucose metabolism mediated by *Kl*Gap2.

In an attempt to find evidence for other/s regulatory factor/s, besides Mcm1, related to transcriptional activation at (−Ca) (or alternatively a repressor at high calcium levels that becomes inactive in absence of calcium) we carried out an in silico analysis of the promoters of these regulated genes using several approaches provided in the RSA tools facilities in order to discover new *cis* elements not described in other regulatory circuits but which were common to the co-regulated genes. However, no statistically significant new regulatory *cis* element was identified. An alternative to explain co-regulation by common *cis* elements is to consider positional effects. Indeed, if the regulated genes are referred to their position in the 16 yeast chromosomes (Table 2), there is an over representation, non-correlated to chromosomal size, in chromosomes X (27 genes), XI (11 genes) and XIII (13 genes). Topological clustering of consecutive or near-neighbouring genes, which are co-regulated, is also observed at high frequency (14 clusters including 40 genes; remarked by ∗ in Table 2). Therefore, it seems plausible that, to some extent, the decay in calcium levels may have an influence over nucleosome positioning or accessibility to the RNA Pol II machinery that enables specific transcription in these regions. One potential candidate to play a role in a general transcriptional regulation, in connection with DNA replication, transcription mediated by RNA Pol II

and RNA Pol III, glucose and amino acid metabolism, is the multifunctional factor Abf1 with is implicated in the clearance of nucleosomes [32]. However, none of the consensus sequences defined for Abf1 binding in other genes are found in the promoters studied.

Genes included in Fig. 2 and Table 2 are only a subset of up-regulated genes at calcium shortage, just including those with ratios higher than 1.8-fold induction. However, our statistical analysis reveals that other genes with minor ratios have still a high statistical significance in transcriptional regulation. Array hybridisation technology usually renders expression differences lower than those obtained by other conventional techniques [33]; therefore it is worthwhile to pay also attention to these genes. An example is *YBR053C*, a gene of unknown function but which encodes a protein with similarities to rat regucalcin, a protein that is able to bind calcium, therefore, it could also play a role in calcium signalling in *S. cerevisiae* since calcium levels regulate their transcription.

In conclusion, the existence of a transcriptional regulation caused by low levels of calcium in yeast has been evidenced by micro array technology and subsequent CA of data. In silico analysis of the promoters from genes related to this response suggests the participation of Mcm1 in this regulation although a positional effect could be also considered.

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