Genome-wide expression screens indicate a global role for protein kinase CK2 in chromatin remodeling

Thomas Barz, Karin Ackermann, Gaelle Dubois, Roland Eils and Walter Pyerin*

Biochemische Zellphysiologie (B0200) and Intelligente Bioinformatiksysteme (H0900), Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany

*Author for correspondence (e-mail: w.pyerin@dkfz-heidelberg.de)

Accepted 7 January 2003
Journal of Cell Science 116, 1563-1577 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00352

Summary

Protein kinase CK2, a vital, pleiotropic and highly conserved serine/threonine phosphotransferase is involved in transcription-directed signaling, gene control and cell cycle regulation and is suspected to play a role in global processes. Searching for these global roles, we analyzed the involvement of CK2 in gene expression at cell cycle entry by using genome-wide screens. Comparing expression profiles of *Saccharomyces cerevisiae* wild-type strains with strains with regulatory or catalytic subunits of CK2 deleted, we found significant alterations in the expression of genes at all cell cycle phases and often in a subunit- and isoform-specific manner. Roughly a quarter of the genes known to be regulated by the cell cycle are affected. Functionally, the genes are involved with cell cycle entry, progression and exit, including spindle pole body formation and dynamics. Strikingly, most CK2-affected genes exhibit no common transcriptional control features, and a considerable proportion of temporarily altered genes encodes proteins involved in chromatin remodeling and modification, including chromatin assembly, (anti-)silencing and histone (de-)acetylation. In addition, various metabolic pathway and nutritional supply genes are affected. Our data are compatible with the idea that CK2 acts at different levels of cellular organization and that CK2 has a global role in transcription-related chromatin remodeling.

Key words: Protein kinase CK2, *Saccharomyces cerevisiae*, Cell cycle, Gene expression, Chromatin remodeling

Introduction

Protein kinase CK2 (also known as casein kinase 2) is a serine/threonine phosphotransferase that occurs ubiquitously in eukaryotes. CK2 is a tetramer, composed of two catalytic subunits, α, and two regulatory subunits, β, formed through stable β-β dimer intermediates that complex with α to constitute the $\alpha_2\beta_2$ holoenzyme (Niefind et al., 2001). Isoforms of these subunits may exist and may play tetramer-independent roles in the cell. Humans have one type of regulatory subunit and two catalytic subunit isoforms (α and α') and thus form $\alpha_2\beta_2$, $\alpha'_2\beta_2$ and $\alpha\alpha'\beta_2$ holoenzymes (Bodenbach et al., 1994), whereas the budding yeast *Saccharomyces cerevisiae* has two catalytic (Cka1 and Cka2) and two regulatory (Ckb1 and Ckb2) subunits that appear to build an obligatory heterotetramer (Glover, 1998). In yeast, deletion of any of the four subunit genes (*CKA1*, *CKA2*, *CKB1* and *CKB2*) individually, and *CKB1* and *CKB2* together, has no overt phenotypic effect in normal media, but such strains exhibit varying degrees of sodium sensitivity. Deletion of *CKA1* and *CKA2* together is lethal, but can be rescued by catalytic subunits of other organisms such as fly and man (Padmanabha et al., 1990; Dotan et al., 2001), demonstrating that protein kinase CK2 is both vital for cells and conserved over large evolutionary distances.

Despite being one of the earliest protein kinases discovered (Burnett and Kennedy, 1954), the biological role of CK2 is still far from being completely characterized. This is mainly a consequence of CK2's marked pleiotropic nature. So far, more than 300 substrates have been identified (Pinna, 2002). The nature of these substrates – predominantly proteins related to transcription and transcription-directed signaling – has led to the assumption that CK2 plays a global role in cell regulation, with roles particularly in gene expression and signal transduction. Many CK2 functions appear to be individually dispensable, but their collective loss is not. On the basis of its vital importance and known interactions with components of stress signaling, growth signaling and survival signaling pathways, a role for CK2 as a survival factor has been proposed (Ahmed et al., 2002).

In *S. cerevisiae*, CK2 is required for G2/M and G1 phases of the cell cycle (Glover, 1998). Previously, we have shown that the cell cycle entry of human cells from G0 and the subsequent early progression through G1 also requires CK2; its perturbation in cultured human cells at either the nucleic acid level (by antisense oligonucleotides) or the protein level (by microinjection of antibodies or substrate peptide analogs) significantly inhibits this process (Pepperkok et al., 1993; Pepperkok et al., 1994; Lorenz et al., 1993; Lorenz et al., 1999). Concomitantly, expression of immediate early genes such as *fos* is suppressed (Pepperkok et al., 1993). The immediate early gene products, for their part, trigger waves of gene expression, reflecting phase-specific hierarchical transcription programs (Iyer et al., 1999). When cells re-enter the cell cycle, they either resume proliferation or go into apoptosis. Because cells are able to remain in G0 for any length of period, re-entry may, in a cell sociological context, have

important effects, including cancer development. CK2 has been linked to such pathophysiological processes (ole-MoiYoi, 1995; Seldin and Leder, 1995; Landesmann-Bollag et al., 2001).

CK2 may carry out its global role in gene expression through common transcriptional features and/or through higher-order structural processes such as nucleosomal organization. The mechanism CK2 uses is not known, but it should be disclosed by comprehensive comparison of CK2-affected genes. We examined the situation at cell cycle entry by using a comparative genome-wide expression analysis of *S. cerevisiae* CK2 subunit deletion strains. The data not only provide a comprehensive overview of CK2-linked gene expression, but also reveal specific contributions of the individual CK2 subunits and identify functional connections. Several CK2-affected genes encode metabolic pathway and spindle pole body (SPB) components as well as cell cycle control proteins such as cyclins and are differentially affected by the respective CK2 subunits. Strikingly, most CK2-linked genes lack common control features, and a significant proportion of temporarily altered genes encode chromatin-remodeling and modification proteins. Together with available data on CK2's association with and phosphorylation of diverse chromatin components and modifiers, our results strongly suggest a global role for protein kinase CK2 in nucleosomal remodeling processes that are particularly important at transition points such as cell cycle (re-)entry.

Materials and Methods

Strains and growth conditions

The CK2 mutant strains *ckb1*Δ *ckb2*Δ [YAPB10-2c, *MAT***a** *CKA1 CKA2 ckb1-Δ1::HIS3 ckb2-Δ1::LEU2* (Bidwai et al., 1995)], *cka1*Δ [JC4-1a, *MAT***a** *cka1-Δ1::HIS3 CKA2 CKB1 CKB2* (Chen-Wu et al., 1988)] and *cka2*Δ [RPG22-1b, *MAT***a** *CKA1 Δcka2::TRP1 CKB1 CKB2* (Padmanabha et al., 1990)] as well as their respective wildtypes (YPH499, *MAT***a** *CKA1 CKA2 CKB1 CKB2* and YPH250, *MAT***a** *CKA1 CKA2 CKB1 CKB2*) were generously provided by C. V. C. Glover (Biochemistry and Molecular Biology, University of Georgia, Athens, GA). All strains were grown in YPD medium at 30°C and shaken at 230 rpm.

α-Factor-based synchronization

Pheromone-based synchronization was performed as previously described (Spellman et al., 1998). Briefly, yeast cells were grown to early log phase (OD_{600} 0.2) and arrested by the addition of α-factor (12 μg/ml), followed by 2 hours of cultivation. After centrifugation, arrest release was effected by resuspension of the cell pellet in fresh (pheromone-free) medium to an OD_{600} of 0.18. During further cultivation samples were taken 0, 7 and 14 minutes after release by adding culture aliquots to sterile ice. Cells were immediately collected by centrifugation, and pellets were flash-frozen in liquid nitrogen. Synchrony was verified by FACS analysis as previously described (Nash et al., 1988).

Sample preparation, hybridization and scanning procedure

The following procedures were performed as previously described (Ackermann et al., 2001). Briefly, total RNA was isolated by applying the hot phenol protocol followed by mRNA concentration using oligo(dT)-cellulose (Ambion). 3 μg of mRNA were reverse transcribed using SuperScript™Choice System for first and second strand cDNA synthesis (Gibco BRL); 1 μg cDNA was transcribed in vitro using biotinylated ribonucleotides (ENZO BioArray™ High Yield™ RNA transcript labeling kit, ENZO Diagnostics). After fragmentation, 15 μg of biotin-labeled cRNA were taken for hybridization to oligonucleotide arrays (YG-S98 Arrays, Affymetrix) at 45°C, 60 rpm for 16 hours. Staining (performing the three-stain procedure including a signal amplification step) and washing steps (applying the protocol EukGE-WS2.v4) were carried out on a GeneChip Fluidics Station 400 (Affymetrix). Arrays were scanned on a HP GeneArray™ scanner (Affymetrix). The resulting image data were analyzed using the GeneChip Expression Analysis software (Micoarray Suite version 4.0; Affymetrix).

Data processing and correspondence analysis

For data analysis, expression values corresponding to an 'absent call' or to a negative value were set to one, followed by uploading data in M-CHIPS [multi-conditional hybridization intensity processing system (Fellenberg et al., 2002)]. This MATLAB-based tool was used for further analysis (normalization and filtering).

For normalization, loglinear regression accounting for affine-linear deviations among the different hybridizations (Beissbarth et al., 2000) was applied. Each hybridization experiment was normalized with respect to the gene-wise median of the control condition (time point 0 of the corresponding wildtype), which was subsequently referred to as the standard. To correct for distortions, the 5% quantile of each hybridization was subtracted initially. As the transcription levels of the majority of genes were unaltered in the different conditions, normalization factors were calculated on the basis of the majority of the spots. In contrast to Beissbarth et al. (Beissbarth et al., 2000), low-intensity signals were kept, and hybridizations were shifted additively back to a more natural range, that is, to the level of the standard (Fellenberg et al., 2001).

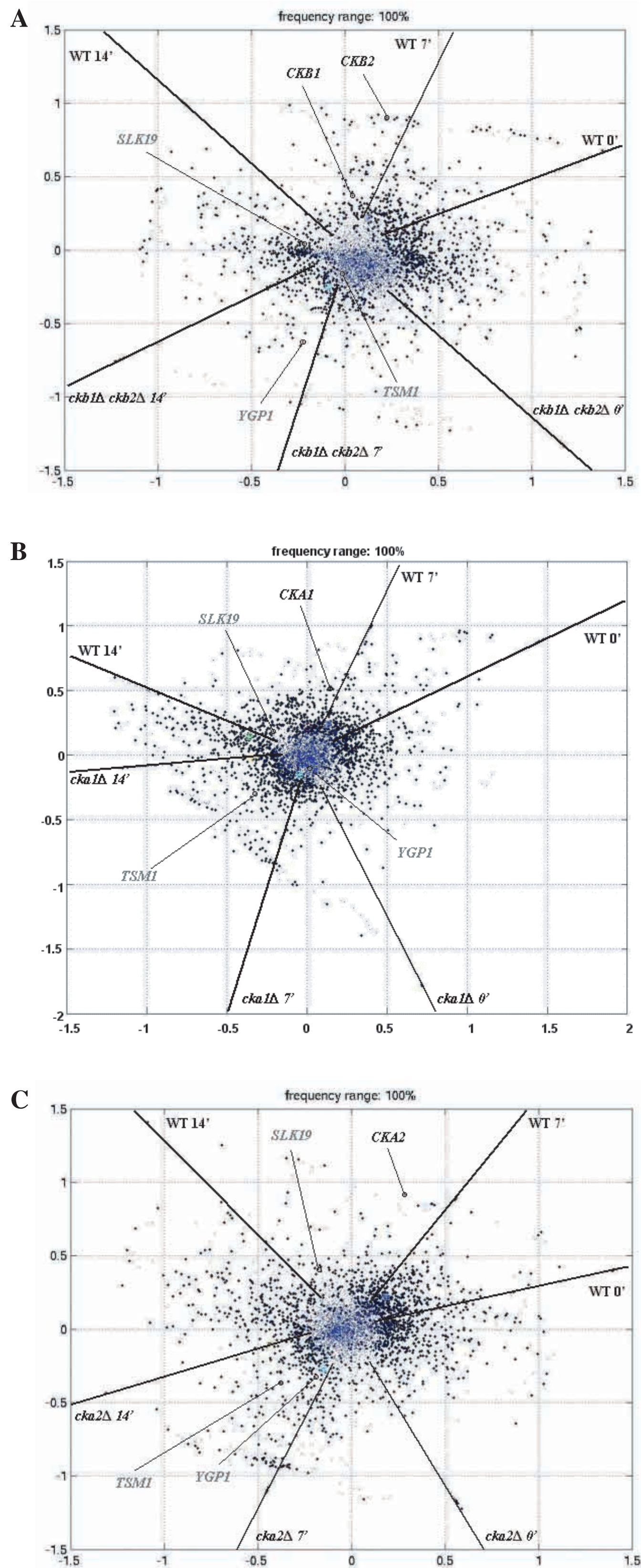
To visualize interdependencies among the high-dimensional data received in two-way contingency tables with rows representing genes and columns representing hybridizations, singular value decomposition (Alter et al., 2000) was used for reducing dimensionality, and the so-called chi-squared distance among the data points was approximated from below. Afterwards, genes and hybridizations were plotted together in a two-dimensional space, where expression specificities are indicated by the distances from the center.

Results

Genetic perturbation of CK2 at cell cycle (re-)entry significantly affects gene expression

In order to identify genes whose expression depends on or is affected by protein kinase CK2 at cell cycle (re-)entry, we performed a comparative genome-wide expression analysis of *S. cerevisiae* CK2 deletion strains and corresponding wildtypes. The deletions were a regulatory CK2 subunit double mutant, strain *ckb1*Δ *ckb2*Δ, and mutants of the individual catalytic subunits, strains *cka1*Δ and *cka2*Δ. All strains, grown to early log phase (OD_{600} 0.2) in order to avoid metabolite and oxygen deficiency effects (DeRisi et al., 1997), were synchronized by α-pheromone treatment (verified by FACS analysis), and transcript profiles were determined at 0, 7 and 14 minutes after pheromone release on oligonucleotide arrays as previously described (Ackermann et al., 2001). According to Spellman et al., gene expressions peaking at 0 to 7 minutes after release are specific for M/G1 phase transition, and those at 14 minutes, for early G1 phase (Spellman et al., 1998). Data were uploaded in M-CHIPS [multi-conditional hybridization intensity processing system (Fellenberg et al., 2002)] and normalized with respect to the gene-wise median of the

standard (time point 0 of the corresponding wildtype) in order to correct distortions caused by background and multiplicative factors so that the different data sets were comparable.



We found various interdependencies between gene expression profiles and CK2 perturbations. By applying correspondence analysis to the data (Fellenberg et al., 2001), the association of individual genes with a specific condition (yeast strain and time point of interest) was determined from their distance from the center and location; the further the distance from the center, the more pronounced the association of the gene with the considered condition (Fig. 1). For instance, *SLK19*, a G1 phase gene whose product is involved in spindle dynamics control, is modestly repressed in the *ckb1*Δ *ckb2*Δ strain but strongly repressed in strains *cka1*Δ and *cka2*Δ and thus only slightly (Fig. 1A) or significantly (Fig. 1B,C) shifted to wild-type areas, respectively. In addition to such strain-specific transcript deviations, time-point-dependent deviations are also indicated for each gene: transcript levels of *YGP1*, for example, a M/G1 phase gene encoding a starvation-induced glycoprotein, were elevated at all time points in strains *ckb1*Δ *ckb2*Δ and *cka2*Δ but not in *cka1*Δ so that the gene is localized at a significant distance from the center along the 7 minutes mutant axis (Fig. 1A,C) or within the center (Fig. 1B), respectively. By contrast, *TSM1*, a G2/M phase gene encoding a TATA-binding protein-associated factor (TAF150), increased transcription at the later time points in strains *cka1*Δ and *cka2*Δ but not *ckb1*Δ *ckb2*Δ, positioning *TSM1* farther from the center, between the 7 and 14 minutes mutant axes (Fig. 1B,C), or within the center, respectively. In summary, correspondence analysis shows CK2-linked expression of various genes at cell cycle entry and indicates specific contributions by the individual CK2 subunits.

By considering the greater than two-fold transcript deviations in at least one of the CK2-deficient strains at one of the time points as significant, we identified 283 genes of the ~900 characterized as cell-cycle-regulated in the literature (Spellman et al., 1998; Cho et al., 1998) as linked to CK2 in their expression (Fig. 2A). Most transcriptional alterations (140) were observed in the regulatory subunit double mutant. Eighty of these were *ckb1*Δ *ckb2*Δ specific, the rest was also altered in the catalytic subunit mutants. In the latter, fewer genes were concerned; 56 and 47 were *cka1*Δ and *cka2*Δ specific, respectively. Regarding time point distribution, the total number of affected genes were comparable at 0 and 7 minutes (157 and 155 genes, respectively) but lower at 14 minutes (128 genes), suggesting stronger CK2 perturbation effects at earlier cell cycle stages (Fig. 2B).

Fig. 1. Correspondence analysis graphs illustrating the dependencies of gene expression profiles during the early cell cycle of CK2 deletion strains. Transcript profiles of α-factor-synchronized yeast strains were determined at 0, 7 and 14 minutes after pheromone release by hybridization to oligonucleotide arrays across the whole yeast genome. Expression specificities of single genes can be detected by their distance from the center and the direction of the corresponding condition. (A) *ckb1*Δ *ckb2*Δ strain compared with the wildtype. (B) *cka1*Δ strain compared with the wildtype. (C) *cka2*Δ strain compared with the wildtype. Note that CK2 mutants were constructed by gene disruption techniques. Thus, although fractional transcription of deleted CK2 subunit genes results in no functioning proteins, weak transcript signals are detected on oligonucleotide arrays, and their distance from the center is not as far as one might expect.

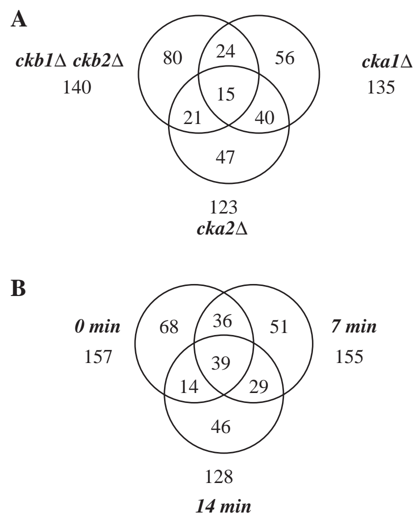


Fig. 2. Cell-cycle-regulated genes affected in early cell cycle expression by perturbation of protein kinase CK2. Significantly deviating expression was defined as having at least a two-fold difference in transcription levels compared with the wildtype. (A) A Venn diagram showing numbers of deviating genes at at least one time point in the different CK2 subunit deletion strains. (B) Time point distribution of genes altered in at least one of the CK2 mutants.

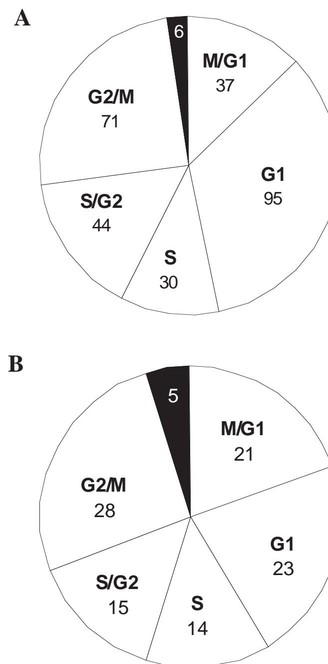


Fig. 3. Phase-specific distribution of altered cell cycle genes in the CK2 mutants. (A) Pie chart showing total numbers of genes altered at cell cycle entry according to peak expression. (B) Number of genes showing expression deviations in asynchronous CK2 mutant cultures according to peak expression. Black sections contain genes peaking at multiple phases.

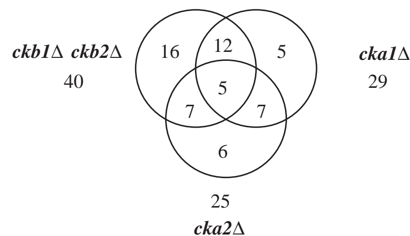


Fig. 4. Cell-cycle-regulated genes showing persistent expression deviations in CK2 deletion strains. Venn diagram showing CK2-mutant-specific distribution of genes altered in the early cell cycle and asynchronous cultures.

CK2-linked genes relate to various cell cycle phases

Surprisingly, the 283 genes affected by CK2 perturbation at cell cycle (re-)entry do not represent a collection of early genes as one might expect from observations with human cultured cells (Lorenz et al., 1993; Pepperkok et al., 1993; Pepperkok et al., 1994). Rather, according to their peak expression (Spellman et al., 1998; Cho et al., 1998), the genes can be assigned to various cell cycle stages. Aside from affected early genes (37 M/G1 phase and 95 G1 phase genes), genes ascribed to S phase (30 genes), S/G2 phase (44 genes), G2/M phase (71 genes) and diverse phases (6 genes) also exhibited altered transcriptional levels (Fig. 3A). For all of these groups, the number of genes deviating in expression remained more or less the same over time, except for G1 phase genes. Their number decreased steadily so that less than half of the alterations at 0 minutes were seen at 14 minutes (data not shown), suggesting particularly strong CK2 requirement for early G1-specific gene expression.

When the CK2 mutant strains were grown as asynchronous permanently cycling cultures (early log phase, OD_{600} 0.46), the transcript levels of 106 cell-cycle-regulated genes were altered. This is a significantly smaller number than that obtained for the cell cycle (re-)entry cultures above. Strikingly, only 23 G1-specific genes were altered (Fig. 3B), indicating that CK2 perturbation in asynchronous cultures does not primarily affect G1-specific genes. G2/M-specific genes form the largest group of affected genes (28 genes); the number of M/G1 genes (21 genes) was similar to that of G1 genes, and 14 and 15 expression alterations were found for S- and S/G2-specific genes, respectively.

By comparing the results from cells synchronized at cell cycle entry with those in permanently cycling cultures, 58 were identified that were altered in both cultures. 40 of these were found in the regulatory CK2 subunit mutant, 16 being *ckb1*Δ *ckb2*Δ specific. In the catalytic CK2 subunit mutants, 29 and 25 genes were altered, and 5 and 6 were *cka1*Δ and *cka2*Δ specific, respectively (Fig. 4). Thus, deletion of the regulatory CK2 subunits not only affects expression of more cell cycle genes than deleting a catalytic subunit, it is also characterized by a significantly stronger persistence. The names, expression and cell cycle phase-association of these 58 persistently altered CK2-linked genes, together with the physiological roles of their respective gene products, are given in Table 1.

More than two thirds of the persistently altered genes exhibited the same deviation characteristics in terms of transcription elevation/repression owing to a certain CK2 mutation in both asynchronous and early cell cycle cultures. For instance, the expression of *PCL2*, a gene encoding a G1 cyclin, was elevated predominantly in the *cka2*Δ mutant, whereas *MIF2*, encoding a centromer protein (the human

Table 1. CK2 perturbation-affected cell cycle genes in early cell cycle and asynchronous CK2 subunit mutant cultures

Gene		*ckb* Δ			*cka1* Δ			*cka2* Δ			Asynchronous			
ORF	Synonym	0	7	14	0	7	14	0	7	14	*ckb* Δ	*cka1* Δ	*cka2* Δ	Gene product
G1 phase genes														
YBR108W		△	-	-	△	-	△	▲	-	-	△	-	▼	has weak similarity to *R. norvegicus* atrophin-1 related protein
YDL124W		-	-	△	-	△	-	-	▲	△	-	-	▲	has similarity to aldose reductase
YDL127W	*PCL2*	-	▽	-	-	-	△	-	△	▲	△	△	▲	G1 cyclin
YDR461W	*MFA1*	-	-	-	▲	△	▼	-	-	▼	▲	-	▽	α-factor mating pheromone precursor
YJL217W		▼	▼	▼	▼	-	-	-	-	-	▼	-	-	has similarity to conserved Vibrio cholerae protein VCA0345
YKL089W	*MIF2*	-	-	▼	-	-	-	-	-	-	▼	-	-	centromere protein
YKL161C		-	▼	▽	▽	▽	-	▽	▼	-	▼	▽	▼	has strong similarity to Ser/Thr-specific protein kinase Slt2
YLR231C		▽	▼	▽	▼	△	△	-	▲	△	▼	-	-	transferase, involved in amino acid metabolism
YLR382C	*NAM2*	△	▽	-	△	▼	-	△	▼	▽	▲	-	-	mitochondrial leucyl tRNA synthetase
YOR372C	*NDD1*	▲	-	△	-	▼	▽	-	-	▽	▲	-	-	transcriptional activator
YPR160W	*GPH1*	▽	-	▽	-	-	-	▲	△	△	-	△	▲	glycogen phosphorylase
S phase genes														
YER091C	*MET6*	▼	▼	▼	▽	-	-	-	-	-	▼	-	▼	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase
YFR030W	*MET10*	▼	▼	▼	-	-	▲	▽	▽	-	▼	△	-	subunit of assimilatory sulfite reductase
YGL184C	*STR3*	▼	▽	▽	-	△	▲	-	△	-	▼	▲	▼	cystathionine β-lyase
YJR137C	*ECM17*	▽	▽	▼	▽	-	▲	▼	-	-	-	▲	-	putative sulfite reductase
YKL001C	*MET14*	▼	▼	▼	-	-	-	-	-	-	▽	▲	-	adenylylsulfate kinase
YPL250C	*ICY2*	-	-	-	-	▲	▲	-	▽	▽	-	▲	-	involved in chromatin organization and nuclear transport
YPL274W	*SAM3*	▼	▽	▼	▽	▽	-	▼	▼	▽	▽	-	▼	S-adenosylmethionine permease
S/G2 phase genes														
YBR104W	*YMC2*	▽	▽	▼	△	-	△	-	-	-	▼	▽	▽	mitochondrial carrier protein
YCL025C	*AGP1*	-	▽	▼	-	-	△	▽	▽	-	▽	-	▼	amino acid permease
YER069W	*ARG5,6*	▽	▽	-	△	▲	▲	-	-	-	△	▲	-	N-acetyl-γ-glutamyl-phosphate reductase and acetylglutamate kinase
YHR006W	*STP2*	-	▼	▼	-	△	△	-	▼	-	▽	-	▼	involved in pre-tRNA splicing
YHR208W	*BAT1*	▼	▼	▼	-	-	-	-	-	-	▼	-	▽	branched-chain amino acid transaminase
YJR048W	*CYC1*	▲	△	-	-	-	-	△	-	-	▲	-	-	iso-1-cytochrome c
YKR039W	*GAP1*	-	▼	▼	-	-	-	-	▽	-	▼	-	▽	general amino acid permease
YOL058W	*ARG1*	▼	-	-	▲	△	-	-	-	-	▼	▲	-	arginosuccinate synthetase
YPR167C	*MET16*	▼	▽	▼	△	-	▲	▽	▼	▼	-	▲	▽	3'phosphoadenylylsulfate reductase
G2/M phase genes														
YAR071W	*PHO11*	▼	▼	▼	▼	▼	-	-	-	-	▼	-	▼	acid phosphatase, secreted
YBR054W	*YRO2*	-	▽	-	▼	-	-	▼	▽	-	-	-	▲	homolog to HSP30 heat shock protein Yro1
YBR093C	*PHO5*	▼	▼	▼	▼	▼	▼	-	▽	-	▼	▽	▽	acid phosphatase, repressible
YDR380W	*ARO10*	▽	▼	▼	-	-	▲	-	-	△	▼	△	▲	unknown function
YFL026W	*STE2*	-	-	-	-	-	△	-	-	▲	▲	-	-	α-factor pheromone receptor
YGL255W	*ZRT1*	-	▼	▼	▼	-	-	▼	▼	▼	▼	-	▼	high-affinity zinc transporter
YGR065C	*VHT1*	▽	▼	▼	-	△	▲	-	-	-	▼	△	-	vitamin H transporter
YHR137W	*ARO9*	▼	▼	▼	-	△	△	△	-	△	▼	▲	▲	aromatic amino acid aminotransferase II
YIL011W	*TIR3*	-	-	▽	▼	-	△	-	-	▲	-	▲	-	cell wall mannoprotein
YJL079C	*PRY1*	△	▲	▲	-	-	-	-	-	-	▲	-	-	similar to plant PR-1 class of pathogen related proteins
YJL157C	*FAR1*	-	▼	▽	-	-	△	-	-	-	▲	-	-	factor arrest protein
YLR413W		-	-	▲	▽	-	△	▽	▽	▲	-	▲	-	hypothetical protein
YML116W	*ATR1*	△	△	△	△	△	▲	△	△	-	▼	△	-	multidrug efflux pump
YML123C	*PHO84*	▽	-	▼	▼	-	-	-	-	-	▼	-	-	inorganic phosphate transporter
YMR058W	*FET3*	▲	▲	▲	-	-	-	▼	▼	▼	▲	-	▽	multicopper oxidase
YPL095C		△	-	△	-	△	-	△	▲	-	▽	-	▲	hypothetical protein
YPR157W		▼	▼	▼	▼	-	△	▼	▼	▽	▲	△	-	hypothetical protein
M/G1 phase genes														
YCR005C	*CIT2*	-	▼	▼	-	-	▲	-	▽	-	▼	▽	▼	non-mitochondrial citrate synthase
YCL027W	*FUS1*	▽	▼	▼	-	△	△	-	-	-	▲	-	▼	MAP kinase involved in pheromone signal transduction
YCL055W	*KAR4*	-	▼	▼	-	-	-	-	-	-	▲	-	▼	transcription factor
YDL181W	*INH1*	▲	▲	▲	-	-	-	▲	▲	-	▲	-	▲	ATPase inhibitor
YHR092C	*HXT4*	-	-	△	▼	▼	-	-	▽	-	▲	▲	▲	high-affinity glucose transporter
YHR094C	*HXT1*	▼	▼	▼	▽	-	-	▽	▼	▼	▼	-	-	high-affinity hexose (glucose) transporter
YIL114C	*POR2*	-	▼	▽	▼	-	-	▼	▼	▼	▽	-	▼	voltage-dependent anion channel
YLR452C	*SST2*	-	▼	▽	-	-	△	-	-	-	▲	-	▼	involved in desensitization to a-pheromone
YNR067C		-	-	-	▽	▲	▲	-	-	-	▼	▽	▽	has similarity to β-glucan-elicitor receptor
YPL014W		-	-	-	▼	▲	-	-	▲	-	▲	-	△	hypothetical protein
YPR002W	*PDH1*	▼	▼	▼	▼	▼	-	▼	▼	▽	▼	▽	▼	involved in propionate metabolism
Genes peaking at multiple phases														
YDR277C	*MTH1*	-	▲	▲	-	▼	△	-	▽	△	▲	▲	▲	repressor of hexose transport genes
YML091C	*RPM2*	△	△	▲	△	▽	-	▲	-	△	▲	-	-	subunit of mitochondrial RNase P
YLR281C		▼	-	▼	-	▽	-	-	-	-	-	-	▲	hypothetical protein

Expression deviations [mutant/wild-type] at 0, 7, and 14 minutes after release from pheromone arrest and in early-log phase cultures, respectively, are indicated by arrowheads: ▲, elevation (>2x); ▼, repression (<0.5x).

Unfilled arrowheads indicate deviation tendencies (>1.5x <2x and >0.5x <0.66x, respectively).

Genes are subdivided according to their peak expression during the cell cycle.

CENP-C homologue), was strongly repressed in the *ckb1*Δ *ckb2*Δ mutant. A particularly interesting situation was provided by the S phase genes. The gene products of all seven continuously altered S phase genes (*MET6*, *MET10*, *MET14*, *STR3*, *ECM17*, *ICY2*, *SAM3*) are involved in methionine biosynthesis and belong to a 20-gene 'MET' cluster peaking coordinately during the cell cycle (Spellman et al., 1998). Remarkably, although repressed in the *ckb1*Δ *ckb2*Δ and *cka2*Δ

mutants, significantly altered MET genes exhibited exclusively elevated transcript levels in the *cka1*Δ mutant. We also noticed this CK2-subunit- and isoform-dependence for the S/G2-specific MET cluster gene *MET16*. In summary, the MET genes represent an excellent example of subunit- and isoform-specific CK2 perturbation effects that are apparently not restricted to early cell cycle expression but rather persist throughout the cell cycle.

ECA39, the mammalian homologue of the S/G2 gene *BAT1*, is regulated by c-Myc and promotes apoptosis in murine cells (Eden and Benvenisty, 1999). Yeast *BAT1,* encoding a branched-chain amino acid transaminase, is probably involved in the regulation of the G1/S transition, as *bat1*Δ strains exhibit elevated growth rates owing to a shortened G1 phase and higher rates of UV-induced mutations (Schuldiner et al., 1996). The persistent *BAT1* repression in the *ckb1*Δ *ckb2*Δ mutant suggests an apoptosis-inhibiting CK2 function. Evidence for a role of CK2 in apoptotic cellular processes not only via gene regulation but also at the protein level is increasing. For instance, Li et al. have recently shown that CK2-mediated phosphorylation is required for activation of a caspase-inhibiting protein in order to prevent apoptosis (2002).

Other S/G2 genes as well as G2/M and M/G1 genes with similar deviation characteristics in early cell cycle and asynchronous cultures are involved in amino acid metabolism and nutrition supply. These include genes for the amino acid permease Gap1, the aromatic amino acid aminotransferase Aro9, the acid phosphatase Pho5 and the propionate metabolism-associated protein Pdh1.

The remaining third of permanently altered genes exhibited diverse deviation characteristics. Several of the genes encode proteins involved in the pheromone signaling pathway, including *FUS1*, *KAR4* and *SST2,* which encode a MAP kinase, a transcription factor, and a GTPase-activating protein, respectively. In the *ckb1*Δ *ckb2*Δ mutant, the genes were strongly repressed during the early cell cycle but elevated in asynchronous culture, whereas in the *cka2*Δ mutant they were unaltered in the early cell cycle and repressed in asynchronous culture.

In contrast to genes associated with the pheromone pathway, MET cluster and nutrition supply, various gene groups that constitute considerable portions of the CK2 perturbation-affected genes at cell cycle (re-)entry (see below) were significantly underrepresented among the genes deviating in permanently cycling CK2 mutant cultures. For example, of ~30 genes linked to chromatin remodeling (CHR) and/or spindle pole body (SPB) organization that exhibited expression deviations at cell cycle (re-)entry, only two genes (*MIF2* and *ICY2*) were altered in asynchronous cultures. Similarly, of at least 15 genes deviating at cell cycle (re-)entry whose products are involved in DNA replication and cell cycle control, only *PCL2* and *NDD1* showed altered transcript levels in asynchronous cultures. This contrast clearly indicates that, although CK2 perturbation considerably affects early cell cycle expression of genes crucial for the (re-)entry, yeast cells are obviously able to compensate for these transcriptional deviations during the course of cell cycle progression.

CK2-linked genes at cell cycle (re-)entry

After subtraction of the 58 permanently altered genes, the remaining 225 genes should more closely reflect CK2-linked genes specifically related to cell cycle (re-)entry. Below, we present all the genes falling into this category, grouped according to when their expression peaked during the cell cycle. These data and the physiological roles of their respective gene products are presented in Table 2.

CK2-linked G1 phase genes

84 cell-cycle-regulated genes are significantly affected by CK2 perturbation at cell cycle (re-)entry peak during G1. Generally, if deviation tendencies are also considered, we found that 90% of altered G1 genes were altered in at least two of the three analyzed CK2 subunit deletion strains. About two thirds of these 84 genes exhibited similar deviation characteristics (repression, elevation) in the CK2 mutants. The rest included genes, such as *HSN1* or *YJR154W*, whose expression was oppositely affected, providing further evidence for a functional specialisation of the catalytic CK2 isoforms and autonomous roles for the regulatory subunits, as already noted for mammalian cells (Lorenz et al., 1999; Vilk et al., 1999). Most of the significantly altered G1-specific genes were repressed. One group of such genes is represented by four members of the 11-gene TOS family (*TOS6, 7, 10, 11*). TOS originally comes from a multicopy suppressor of DNA topoisomerase II (TopII) mutants, named *TOS1* (Thomas et al., 1991). TopII is regulated by CK2-mediated phosphorylation (Alghisi et al., 1994); however, as long as the biological function of the TOS gene family is not fully understood, a possible connection to CK2 remains speculative.

Because of their temporal coregulation with *CLN2* transcription, 76 G1-specific genes peaking at mid-G1 have been categorized into a CLN2 cluster (Spellman et al., 1998), which contains many genes involved in DNA replication and other important cell cycle functions. Collectively, we found 14 altered CLN2 cluster genes, including *CDC45*, *RNR1* and *CLN2* itself. Several CLN2 cluster genes are induced by the transcription factors MBF and/or SBF (Spellman et al., 1998). Six of the 21 known MBF-regulated genes and 10 of the 24 SBF-regulated genes, as determined by Simon et al. (Simon et al., 2001), were altered; however, we did not observe a common deviation pattern. The related heterodimers SBF (Swi4/Swi6) and MBF (Mbp1/Swi6) provide crucial G1-specific transcriptional regulation in the yeast cell cycle. One of their main functions is the transcriptional activation of the G1/S cyclin genes *CLN1* and *CLN2*. SBF and MBF clearly do not work alone; the list of factors that influence SBF and MBF activities or cooperate with them is growing. Cdc68, a general transcriptional regulator, is an activator of their components Swi4 and Swi6 (Lycan et al., 1994) and has been identified by Glover as a high probability CK2 substrate relevant to cell cycle progression (Glover, 1998). Recently, Cdc68 has been found to be associated with all four CK2 subunits in yeast (Gavin et al., 2002; Ho et al., 2002). Possible phosphorylation of Cdc68 by protein kinase CK2 might explain the polymorphic deviation patterns for SBF/MBF-regulated genes in the CK2 mutants, because mutations in *CDC68* can repress or activate the regulation of transcription (Wittmeyer et al., 1997).

During G1, the SPB replicates and expression of several

SPB components consistently peaks at this stage (Spellman et al., 1998). A remarkably high number of G1 genes affected in our study encode proteins required for SPB assembly or spindle movement control: the SPB component genes *NUF1* and *CNM67* exhibited divergent expressional alteration characteristics in the diverse CK2 mutants, whereas *HCM1* and *PAC11*, whose gene products are needed for SPB assembly, were primarily repressed. Repression was also observed for *SLK19, IPL1* and *KAR3*, which encode proteins participating in spindle dynamic control. By contrast, transcript levels of *SMC1* and *CSM2*, whose products are also directly or indirectly involved in chromosome segregation processes (*CSM2* in meiosis), were exclusively elevated. Thus, in the early cell cycle, CK2 perturbation causes complex expressional changes in spindle-function-associated genes.

Three of the affected G1 genes are involved in chromatin

Table 2. CK2-perturbation-affected cell cycle genes in early cell cycle CK2 subunit mutant cultures

Gene		***ckb* Δ**			***cka1* Δ**			***cka2* Δ**			
ORF	Synonym	0	7	14	0	7	14	0	7	14	Gene product
G1 phase genes											
YBL009W		▲	-	-	-	-	-	△	-	-	has strong similarity to DNA-damage-responsive Alk1
YBR007C		-	▽	-	▼	-	-	-	▽	-	hypothetical protein
YBR071W		-	-	-	▼	-	-	-	-	-	hypothetical protein
YBR088C	*POL30*	▽	-	-	-	-	-	-	▲	-	profilerating cell nuclear antigen
YBR089W		-	-	-	-	▲	▲	▲	▲	▲	hypothetical protein
YCL042W		▲	-	-	▽	-	-	-	▽	-	hypothetical protein
YCL061C	*MRC1*	▽	-	▽	-	-	-	-	▼	-	transcription factor, required for replication checkpoint
YCR065W	*HCM1*	△	-	-	▽	-	▽	-	▼	-	transcription factor, involved in regulation of spindle pole body assembly
YDL093W	*PMT5*	-	▼	-	▽	-	-	-	-	-	dolichyl phosphate-D-mannose:protein O-D-mannosyltransferase
YDL119C		▽	▼	▽	-	-	-	-	-	-	has similarity to bovine Graves disease carrier protein
YDR113C	*PDS1*	△	△	-	▼	-	-	▼	-	-	42 kDa nuclear protein, putative anaphase inhibitor
YDR356W	*NUF1*	-	-	-	▼	▼	-	▲	-	△	component of the spindle pole body
YDR383C		-	-	-	△	▲	-	-	-	-	has weak similarity to *S. pombe* paramyosin
YDR440W	*PCH1*	▲	▲	-	▲	-	-	△	-	-	putative ATPase
YDR488C	*PAC11*	-	▽	▽	▼	▽	-	▽	▽	-	required in the absence of Cin8 for mitotic spindle assembly
YDR528W	*HLR1*	-	-	-	△	-	-	▲	-	-	has similarity to Lre1
YEL047C	*FRDS1*	▽	-	-	▼	▽	-	▽	▽	-	soluble fumarat reductase
YER001W	*MNN1*	-	▽	▽	▼	▼	▽	-	-	-	α-1,3-mannosyltransferase
YER070W	*RNR1*	▼	-	-	▼	-	-	▽	-	-	ribonucleotide reductase
YER124C		-	△	△	-	-	▲	△	-	-	has weak similarity to *Dictyostelium* WD40 repeat protein 2
YER152C		-	-	-	▼	-	-	-	-	-	has weak similarity to *E. coli* hypothetical protein f470
YFL008W	*SMC1*	-	-	-	▲	△	-	△	▲	-	coiled-coil protein involved in chromosome structure or segregation
YGL027C	*CWH41*	-	-	-	-	-	-	△	-	▲	type II integral membrane N-glycoprotein located in the ER
YGL028C	*SCW11*	-	-	-	-	▲	△	△	-	-	has similarity to glucanase
YGL038C	*OCH1*	▲	-	△	-	△	-	△	▲	△	membrane-bound mannosyltransferase
YGL089C	*MFa2*	▼	▼	▼	▼	-	-	▽	▼	▽	α mating factor
YGR152C	*RSR1*	-	▽	▽	▼	-	-	-	-	-	GTP-binding protein of the ras superfamily
YGR153W	*TOS10*	▼	▼	▼	▼	-	-	▼	-	▽	hypothetical protein
YGR238C	*KEL2*	-	-	-	-	▽	▽	-	▼	▽	protein containing kelch repeats
YHL050C		-	▼	▼	▲	-	△	▲	▼	-	has strong similarity to subtelomerically encoded proteins
YHR038W	*KIM4*	-	▼	▼	-	-	-	-	-	▽	putative mitochondrial ribosome recycling factor
YHR067W		▽	▼	▼	-	-	-	-	-	▽	putative helicase
YHR110W	*ERP5*	-	▽	▼	-	-	▽	-	-	-	has similarity to human gp25L2 protein
YHR123W	*EPT1*	▽	▼	▽	▼	-	△	-	-	△	sn-1,2-diacylglycerol ethanolamine- and cholinephosphotranferase
YHR127W	*HSN1*	▼	▼	▼	▲	-	-	-	△	-	high copy suppressor of N34 dominant-negative allele of SEC4
YHR143W		-	▽	▼	-	-	-	-	-	-	has weak similarity to Aga1
YHR154W	*ESC4*	▼	▽	▼	▼	-	-	-	△	△	establishes silent chromatin
YHR218W		-	-	▼	△	-	△	△	▽	△	has strong similarity to subtelomerically encoded proteins
YIL132C	*CSM2*	▲	▽	-	▲	▲	-	▲	△	-	involved in chromosome segregation
YIL140W	*SRO4*	-	-	-	▼	-	-	-	-	△	localizes to the plasma membrane
YJL019W		-	-	-	△	-	-	-	▼	-	has weak similarity to hypothetical *C. elegans* protein
YJL072C		-	▼	-	▲	-	△	-	-	-	has similarity to hypothetical *S. pombe* protein SPBC725.13c
YJL115W	*ASF1*	▽	-	▽	▲	△	-	-	-	-	anti-silencing protein
YJL187C	*SWE1*	▲	-	-	-	-	▽	-	-	-	protein kinase homolog
YJR154W		▼	▼	▼	△	▲	△	-	▼	-	has similarity to *Gibberella moniliformis* Fum9
YJR155W	*AAD10*	▼	▼	-	-	-	▲	-	-	△	has strong similarity to aryl-alcohol dehydrogenase
YKL045W	*PRI2*	-	-	-	▼	▽	-	▼	▽	-	p58 polypeptide of DNA primase
YKL103C	*LAP4*	▲	▲	▲	-	-	-	-	-	△	vacuolar aminopeptidase ysc1
YLL012W		-	-	-	▼	-	-	▽	-	-	has similarity to triacylglycerol lipases
YLL067C		-	▼	▽	▼	▲	-	▲	-	-	has strong similarity to subtelomerically encoded proteins
YLR032W	*RAD5*	▲	-	-	-	-	-	-	-	-	putative ATPase/DNA helicase
YLR103C	*CDC45*	-	-	-	▲	-	-	▲	-	-	chromosomal DNA replication initiation protein
YLR286C	*CTS1*	-	-	△	-	-	▲	-	-	▲	endochitinase
YLR381W	*MLP1*	-	-	-	▼	-	-	-	▽	-	hypothetical protein
YML027W	*YOX1*	▲	▽	-	▼	-	-	▲	△	-	homeobox-domain-containing protein

Continued on p. 1570

Table 2. Continued

Gene		*ckb* Δ			*cka1* Δ			*cka2* Δ			
ORF	Synonym	0	7	14	0	7	14	0	7	14	Gene product
G1 phase genes											
YML102W	*CAC2*	-	-	-	-	-	-	△	△	▲	p60 subunit of the yeast chromatin assembly factor-I
YMR179W	*SPT21*	▲	-	-	-	-	-	▼	-	-	non-specific DNA-binding protein
YNL169C	*PSD1*	-	-	-	△	△	-	▲	-	-	phosphatidylserine decarboxylase 1
YNL181W		-	-	-	-	▼	-	-	▼	-	has similarity to hypothetical *S. pombe* protein
YNL225C	*CNM67*	-	▲	-	▽	▽	-	▼	-	-	component of spindle pole body
YNL300W	*TOS6*	-	▼	▽	▼	-	-	-	▲	-	has similarity to Mid2
YNL303W		-	△	-	△	▲	-	△	▲	△	hypothetical protein
YNL304W	*YPT11*	△	-	-	-	-	△	-	▲	▲	has similarity to Ypt1 and rab GTP-binding proteins
YNL310C		▽	▽	▼	△	-	-	-	-	-	has similarity to *S. pombe* hypothetical protein SPAC24H6.02c
YNL312W	*RFA2*	-	-	-	▼	▽	-	-	-	▼	subunit 2 of replication factor RF-A
YOL007C	*CSI2*	-	▼	▽	△	-	-	△	△	-	putative structural component of the chitin synthase 3 complex
YOL016C	*CMK2*	-	▼	▽	-	-	△	-	-	-	calmodulin-dependent protein kinase
YOL017W		▼	▽	-	▼	▼	-	▼	▽	-	has similarity to Yfr013w
YOL019W	*TOS7*	-	▼	▽	▼	▼	-	▽	▼	-	has similarity to Rim9
YOL090W	*MSH2*	-	-	-	▽	▽	-	▽	▼	-	DNA mismatch repair protein
YOL094C	*RFC4*	▼	▼	▼	▽	-	-	-	-	-	subunit 4 of replication factor C
YOR075W	*UFE1*	-	▽	-	△	-	▽	-	-	▼	syntaxin of the ER
YOR176W	*HEM15*	-	-	-	▼	-	-	-	-	-	ferrochelatase (protoheme ferrolyase)
YOR195W	*SLK19*	-	▽	-	-	▼	-	▼	▼	▽	possible leucine zipper, involved in control of spindle dynamics
YOR248W	*TOS11*	-	-	▽	▼	▽	△	△	-	▲	hypothetical protein
YOR308C	*SNU66*	-	-	-	-	-	-	-	▼	▽	component of U4/U6.U5 snRNP
YOR321W	*PMT3*	-	-	▽	▼	-	-	-	-	-	dolichyl phosphate-D-mannose:protein O-D-mannosyltransferase
YPL057C	*SUR1*	-	-	-	▼	-	-	▽	▼	▽	transferase, involved in fatty acid metabolism
YPL163C	*SVS1*	▲	-	▼	▽	-	-	▲	▲	-	serine and threonine rich protein.
YPL208W		-	-	-	-	▼	▽	-	-	-	has similarity to Yhl039w
YPL209C	*IPL1*	-	▼	-	-	-	-	-	▽	▽	protein kinase of mitotic spindle
YPL256C	*CLN2*	▽	▽	▼	▽	-	-	-	△	-	G(sub)1 cyclin
YPR141C	*KAR3*	▽	▼	▼	-	-	-	-	-	-	kinesin-like nuclear fusion protein
YPR174C		-	-	▼	▽	▽	-	▽	▽	▽	has weak similarity to Nbp1
S phase genes											
YAL067C	*SEO1*	▼	▼	-	▼	▽	-	▼	-	△	suppressor of sulfoxyde ethionine resistance
*YBL002W**	*HTB2**	-	-	▼	-	-	-	△	△	-	histone H2B*
YDR247W		-	▼	-	▼	-	-	▼	-	▼	hypothetical protein
YDR252W	*BTT1*	-	▽	-	-	△	▽	-	-	▼	β-subunit of the nascent-polypeptide-associated complex (NAC)
YDR261C	*EXG2*	-	▽	-	▼	▽	-	▽	▽	-	exo-1,3-β-glucanase
YEL060C	*PRB1*	-	-	-	▼	▽	-	-	▽	-	vacuolar protease B
YFR038W		-	▽	-	-	▽	-	-	▼	-	hypothetical protein
YGL125W	*MET13*	▼	▼	▼	-	-	▲	▼	▼	▽	putative methylenetetrahydrofolate reductase (mthfr)
YGR099W	*TEL2*	-	-	-	-	-	-	△	▲	-	telomere-binding protein
YGR113W	*DAM1*	-	-	▽	▲	-	-	▲	▽	-	microtubule-binding protein required for mitotic spindle integrity and kinetochore function
YHR030C	*SLT2*	▽	▼	▽	-	-	-	-	-	-	MAP kinase
YHR098C	*SFB3*	▼	▼	-	-	-	-	△	△	-	binds to Sed5 and Sec23
YIL074C	*SER33*	-	-	-	▼	-	△	-	-	△	phosphoglycerate dehydrogenase catalyzes first step in serine biosynthesis
YIR017C	*MET28*	▽	▼	▼	▲	△	△	▽	-	-	transcriptional activator of sulfur amino acid metabolism
YKL008C	*LAC1*	-	-	▼	-	△	-	-	-	-	Lag1 cognate homolog
YKL065C	*YET1*	-	-	▼	-	-	-	-	-	-	endoplasmic reticulum 25 kDa transmembrane protein
YLR045C	*STU2*	▲	▲	-	-	-	-	-	-	-	may play a role in organization of microtubule ends at spindle pole bodies
YLR126C		-	▼	▼	-	-	▼	▽	▼	▼	hypothetical protein
*YNL030W**	*HHF2**	-	△	▼	-	-	-	△	△	-	histone H4*
YOR284W		-	▼	▽	△	△	▽	-	▲	-	hypothetical protein
YPL016W	*SWI1*	-	-	△	▼	-	-	-	▼	-	zinc-finger transcription factor involved in chromatin remodeling
YPL054W	*LEE1*	-	-	-	▼	-	-	-	-	-	hypothetical protein
YPR159W	*KRE6*	-	▽	-	-	-	▲	-	-	△	putative β-glucan synthase
S/G2 phase genes											
YBR043C	*AQR2*	-	-	-	-	△	▲	-	-	-	multidrug resistance transporter
YBR243C	*ALG7*	△	△	△	▼	-	-	-	-	-	UDP-N-acetyl-glucosamine-1-P transferase (GPT)
YBR256C	*RIB5*	-	-	-	△	△	▲	-	-	-	riboflavin synthase α-chain
YCR084C	*TUP1*	-	-	△	▼	-	-	-	-	-	general transcriptional repressor
YDL198C	*YHM1*	-	-	-	△	▲	-	-	-	-	putative mitochondrial carrier protein
YDR150W	*NUM1*	-	△	▲	▲	-	-	▲	▲	△	involved in microtubule organization
YDR302W	*GPI11*	-	▼	-	-	-	-	-	-	-	phosphoethanolamine N-methyltransferase
YDR464W	*SPP41*	-	-	-	▼	-	-	-	▽	-	negative regulator of prp spliceosome genes
YGL216W	*KIP3*	▲	△	△	-	-	-	-	-	△	kinesin-related protein
YGR138C	*TPO2*	-	-	-	▼	-	-	-	-	-	polyamine transport protein
YHR086W	*NAM8*	-	▼	▽	▽	▽	-	-	▼	-	U1 snRNP protein

Table 2. Continued

Gene		*ckb* Δ			*cka1* Δ			*cka2* Δ			
ORF	Synonym	0	7	14	0	7	14	0	7	14	Gene product
S/G2 phase genes											
YHR146W	*CRP1*	▼	▽	▼	-	-	-	-	-	-	cruciform DNA-binding protein 1
YHR178W	*STB5*	▽	▽	▽	▼	-	-	-	▼	-	transcription factor involved in xenobiotic response
YIL123W	*SIM1*	-	-	-	▼	-	-	▽	-	-	possibly involved in control of DNA replication
YIL131C	*FKH1*	▲	△	△	-	-	-	△	-	-	transcription factor involved cell cycle control
YIL138C	*TPM2*	-	-	▽	△	▲	-	-	-	▼	tropomyosin isoform 2
YJL060W	*BNA3*	△	-	▲	▼	▽	-	▼	▼	-	arylformamidase
YJL099W	*CHS6*	-	-	-	-	-	-	-	▼	-	involved in chitin biosynthesis
YJL134W	*LCB3*	-	▽	▽	-	-	▲	△	-	-	putative plasma membrane transporter
YKL004W	*AUR1*	-	-	-	▼	-	-	-	-	-	inositol phosphoceramid synthase
YKL035W	*UGP1*	-	-	▽	▼	-	-	-	-	-	UDP-glucose pyrophosphorylase
YKL048C	*ELM1*	-	-	-	△	-	-	▲	▽	-	protein kinase
YKL049C	*CSE4*	-	-	▽	▲	-	▽	-	-	▽	similar to histone H3 and to human centromere protein CENP-A
YLR437C		▽	▽	▼	-	-	-	-	-	-	hypothetical protein
YMR003W		▽	-	▼	-	▽	▽	-	▼	-	hypothetical protein
YMR198W	*CIK1*	▽	▼	▼	-	▲	▲	-	-	-	spindle-pole-body- associated protein
YMR215W	*GAS3*	▲	▲	▲	-	-	-	-	-	-	hypothetical protein
YNL197C	*WHI3*	-	-	-	▼	-	-	-	▼	-	putative RNA binding protein involved in regulation of cell size
YOR274W	*MOD5*	-	-	-	▼	▼	-	▽	▼	-	transfer RNA isopentenyl transferase
YOR324C		-	△	-	-	-	△	-	-	▼	hypothetical protein
YPL116W	*HOS3*	-	-	-	-	▽	-	-	▼	△	histone deacetylase
YPL128C	*TBF1*	-	-	-	▼	-	-	-	▼	▽	TTAGGG-repeat-binding factor
YPL133C	*RDS2*	▲	-	-	-	-	-	-	-	-	transcription factor involved in xenobiotic response
YPL141C		-	▽	-	▽	▽	-	▼	▼	▼	hypothetical protein
YPL264C		▼	▼	▼	-	▲	▲	▼	▼	▼	hypothetical protein
G2/M phase genes											
YAL040C	*CLN3*	-	-	-	▼	-	-	▽	▼	-	G1 cyclin
YBR094W		-	-	-	▼	▼	-	▽	▼	▽	hypothetical protein
YBR200W	*BEM1*	-	-	-	-	-	-	-	▼	-	bud emergence mediator
YCL037C	*SRO9*	-	△	-	△	▽	△	-	▼	-	may be involved in organization of actin filaments
YCL038C	*AUT4*	-	-	-	▼	-	-	▼	▽	▽	essential for breakdown of autophagic vesicles in the vacuole
YCL065W		-	△	-	-	▲	-	△	▲	△	hypothetical protein
YCR042C	*TSM1*	-	△	-	△	▲	▲	▲	▲	▲	TATA binding protein-associated factor (TAF150)
YDL037C		-	▼	▽	-	▲	-	△	-	▼	hypothetical protein
YDL048C	*STP4*	▽	-	-	▼	▼	-	▼	▼	-	involved in pre-tRNA splicing
YDL138W	*RGT2*	▽	▼	▼	-	-	-	-	▽	-	glucose permease
YDR033W	*MRH1*	-	-	▲	-	-	-	▽	▼	-	membrane protein
YEL025C	*SRI1*	▽	-	-	▼	-	-	-	▽	-	Swi/SNF and RSC interacting protein 1
YEL065W	*SIT1*	-	▽	▽	△	-	-	▽	▼	▼	ferrioxamine B permease
YFR039C		-	-	-	-	-	-	▲	-	-	hypothetical protein
YGL116W	*CDC20*	-	-	-	-	-	▲	-	-	-	anaphase-promoting complex (APC) subunit
YGL201C	*MCM6*	▼	-	△	△	△	-	▲	▲	-	ATP-dependent DNA helicase
YGL209W	*MIG2*	▼	-	-	▽	-	-	▼	▼	-	zinc finger protein, transcriptional repressor
YGR035C		-	▽	▽	△	▲	-	△	▲	-	hypothetical protein
YGR143W	*SKN1*	△	-	▲	-	-	△	-	▽	-	putative type II membrane protein
YGR284C	*ERV29*	-	-	-	▼	-	-	-	-	-	ER vesicle protein (29 kDa)
YHL026C		-	▽	▼	-	-	-	-	-	-	hypothetical protein
YHL040C	*ARN1*	△	▽	-	▼	-	▽	▼	▼	▼	siderochrome iron transporter
YHR023W	*MYO1*	▽	▲	-	▽	-	△	▽	▽	-	class II myosin
YHR029C		-	▼	▽	▲	▲	▲	-	-	▼	hypothetical protein
YHR151C		▽	▼	▽	-	-	-	-	-	-	hypothetical protein
YHR152W	*SPO12*	▼	▼	▼	-	-	-	-	-	▽	putative positive regulator of exit from M phase
YHR205W	*SCH9*	▼	▽	▽	-	-	-	-	-	-	protein kinase involved in growth control
YIL056W		-	▼	▼	-	-	△	-	-	▽	hypothetical protein
YIL119C	*RPI1*	-	-	▲	-	▲	▽	-	▲	▲	inhibitor of ras
YIL162W	*SUC2*	-	-	▲	▽	-	-	-	▼	-	invertase (sucrose hydrolyzing enzyme)
YJR092W	*BUD4*	-	-	▼	-	-	-	-	-	▽	co-assembles with Bud3p at bud sites
YKL043W	*PHD1*	-	-	-	-	▽	-	-	▼	-	putative transcription factor
YKL044W		-	-	-	△	△	△	△	▼	-	hypothetical protein
YKL172W	*EBP2*	-	-	▼	-	-	-	▽	▽	-	EBNA1-binding protein homolog (nucleolar)
YLR098C	*CHA4*	▲	-	▲	-	-	-	-	-	△	
YLR347C	*KAP95*	-	-	△	▼	▽	-	▽	▼	▽	karyopherin β homolog (95 kDa)
YLR353W	*BUD8*	▲	-	△	△	△	△	▲	▲	△	involved in bud site selection
YML050W		▲	-	-	△	△	-	△	△	△	hypothetical protein

Table 2. Continued

Gene		ckb Δ			cka1 Δ			cka2 Δ			
ORF	Synonym	0	7	14	0	7	14	0	7	14	Gene product
G2/M phase genes											
YML116W	*ATR1*	△	△	△	△	△	▲	△	△	-	multidrug efflux pump
YML119W		-	-	-	▼	▼	-	▽	▽	-	hypothetical protein
YML120C	*NDI1*	▲	△	▲	▽	▽	-	-	-	-	NADH dehydrogenase (ubiquinone)
YMR031C		-	▲	▲	-	-	-	-	-	-	hypothetical protein
YMR145C	*NDE1*	-	-	▲	▽	-	-	-	-	-	NADH dehydrogenase
YNL057W		▼	-	-	▲	△	-	▲	▼	-	hypothetical protein
YNL058C		-	-	-	▼	-	-	▽	▽	-	hypothetical protein
YOL014W		▽	▼	▽	▽	▼	▼	▼	▼	▼	hypothetical protein
YOL119C	*MCH4*	-	-	-	-	-	▲	-	-	-	homolog to monocarboxylate permease
YOL132W	*GAS4*	-	▼	▼	▼	-	-	▲	-	-	hypothetical protein
YOL150C		-	△	△	-	△	△	▲	▲	▲	hypothetical protein
YOR023C	*AHC1*	△	△	-	▲	-	-	▲	-	△	component of Ada histone acetyltransferase complex
YOR129C		▼	▽	▼	-	-	-	▲	-	-	hypothetical protein
YPL058C	*PDR12*	▽	▼	-	▼	-	△	▼	▼	▼	multidrug resistance transporter
YPL242C	*IQG1*	△	△	▲	-	-	-	-	-	-	probable regulator of morphogenesis
YPL265W	*DIP5*	-	▼	▼	△	▽	-	-	▼	▽	dicarboxylic amino acid permease
YPR128C	*ANT1*	-	▽	-	▽	-	▲	-	-	△	adenine nucleotide transporter
M/G1 phase genes											
YBL061C	*SKT5*	-	▽	-	-	▽	-	-	▼	-	protoplast regeneration and killer toxin resistance protein
YBR204C		-	-	-	▽	▲	▽	-	▲	▽	weak similarity to peroxisomal serine-active lipase
YBR296C	*PHO89*	▼	▼	▼	▼	▽	-	-	-	-	high-affinity phosphate transporter
YCL040W	*GLK1*	-	-	▲	▽	-	-	-	-	-	glucokinase
YDR157W		-	-	-	-	▲	-	-	▲	▲	hypothetical protein
YDR191W	*HST4*	△	-	-	▲	-	△	△	△	-	histone deacetylase
YIL009W	*FAA3*	-	-	▲	-	-	-	△	-	△	acyl CoA synthase
YJL044C	*GYP6*	-	-	△	-	-	-	△	▼	-	GTPase-activating protein
YJR004C	*SAG1*	▼	▽	-	▼	-	-	-	-	-	α-agglutinin
YKL163W	*PIR3*	-	-	-	▼	-	-	▽	▼	-	protein containing tandem internal repeats
YKL178C	*STE3*	▽	▽	▼	▽	-	-	-	-	-	α factor receptor
YKL185W	*ASH1*	-	△	▲	▽	-	-	-	-	-	negative regulator of HO transcription
YLR040C		-	-	-	▼	-	-	▽	-	▽	has weak similarity to hypothetical protein Yil011w
YLR273C	*PIG1*	-	-	▲	▼	-	-	-	-	-	putative type 1 phosphatase regulatory subunit
YMR246W	*FAA4*	-	-	-	-	-	▲	-	-	▲	long-chain fatty acid-CoA ligase and synthetase 4
YMR256C	*COX7*	-	△	-	-	△	-	△	▲	-	subunit VII of cytochrome c oxidase
YNL078W	*JIP1*	▽	▼	▼	▽	-	△	-	-	△	hypothetical protein
YNL160W	*YGP1*	▲	▲	▲	-	-	-	△	▲	△	glycoprotein synthesized in response to nutrient limitation
YNL173C	*MDG1*	-	-	-	▼	▽	-	▽	▼	▽	GTP-binding protein of pheromone response pathway
YNL327W	*EGT2*	▼	-	▽	▼	△	-	-	-	-	cell cycle regulation protein
YOL101C		-	△	-	-	▲	▲	-	△	▲	has similarity to Yol002c and Ydr492w
YOR052C		-	▽	-	-	▽	-	-	▼	-	has similarity to hypothetical *S. pombe* protein
YOR065W	*CYT1*	▲	△	▲	▽	-	-	-	-	-	cytochrome c1
YOR317W	*FAA1*	-	-	-	▽	▽	-	-	▼	-	long-chain fatty acid-CoA ligase
YPL066W	*VPT 28*	-	-	△	-	-	-	-	-	▲	hypothetical protein
YPR204W		-	-	-	△	△	△	△	▲	△	has strong similarity to subtelomerically encoded proteins
Genes peaking at multiple phases											
YKL092C	*BUD2*	▲	▲	△	△	▼	-	-	▽	▼	GTPase-activating protein
YJR159W	*SOR1*	▼	▼	▼	▽	-	-	-	-	-	sorbitol dehydrogenase
YHR031C	*RRM3*	▽	▼	▼	-	-	-	-	-	-	DNA helicase

Expression deviations (mutant/wildtype) at 0, 7 and 14 minutes after release from pheromone arrest are indicated by arrowheads as in Table1.
Genes are subdivided according to their peak expression during the cell cycle.
*Histone mRNA has no polyA-tail.
Because mRNA concentration was performed by oligo-dT-cellulose and oligo-dT-primers were used for reverse transcription (see Materials and Methods), histone gene expression signals are not representative.

remodeling: *CAC2,* encoding a chromatin assembly factor subunit, had elevated expression in the *cka2*Δ strain, whereas *ESC4* and *ASF1*, encoding proteins with chromatin silencing and anti-silencing functions, respectively, exhibited divergent expression deviations depending on the CK2 subunit(s) deleted. Recently, *ASF1* deletion in yeast has been reported to induce cell death with apoptotic features (Yamaki et al., 2001). An interpretation of the *ASF1* transcript profiles (slightly repressed in *ckb1*Δ *ckb2*Δ; significantly elevated in *cka1*Δ) in this context seems difficult.

CK2-linked S phase genes

In common with the G1 genes, we found that most of the 23 S phase genes that show significant deviation in expression are repressed. In the *ckb1*Δ *ckb2*Δ strain, only a single gene, *STU2*,

whose product may play a role in organization of microtubule ends at SPBs, exhibited significantly elevated transcript levels. In the catalytic subunit mutants, the repression:elevation distribution was more balanced. In addition to *STU2*, another microtubule/SPB-associated S phase gene, *DAM1*, which encodes a protein required for spindle integrity and kinetochore function, was altered (elevated at 0 minutes in both *cka1*Δ and *cka2*Δ strains). This is consistent with the diverse expression deviations of spindle-function-linked G1 genes (see above). In common with the G1 group, certain S phase genes that are significantly affected by CK2 perturbation, such as *SWI1* and *TEL2*, are associated with chromatin-remodeling processes. In addition to the eight MET cluster genes also altered in asynchronous cultures (see above), we identified another three MET genes (*MET13*, *SER33* and *MET28*) exhibiting virtually the same isoform/subunit-specific expression deviations (repression in *ckb1*Δ *ckb2*Δ and *cka2*Δ, elevation in *cka1*Δ). For these genes, the corresponding deviation tendencies were also observed in the asynchronous culture (data not shown).

CK2-linked S/G2 phase genes

35 S/G2-specific genes exhibited CK2-perturbation-linked expression deviations at cell cycle (re-)entry and again repression was the major deviation. As before, genes linked to SPB/microtubule organization, such as *KIP3* (encoding a kinesin-related protein) and *CIK1* (encoding a SPB-associated protein), and genes whose products are involved in chromatin remodeling, such as *HOS3* (encoding a histone deacetylase) and *CSE4* (encoding a CENP-A homolog), exhibited diverse deviation characteristics. In this context, expression of *FKH1*, encoding a forkhead transcription factor involved in cell cycle control, was continuously elevated in the *ckb1*Δ *ckb2*Δ strain, but unaltered in *cka1*Δ and slightly elevated (only at 0 minutes) in *cka2*Δ strains, respectively. Fkh1 target gene expression is high in virtually all cell cycle phases but primarily in G2/M (Simon et al., 2001). Interestingly, Fkh1 binds to promoters of various chromatin-structure-associated genes, including the S-specific *TEL2* gene (see above) and *HOS3* (Simon et al., 2001). Deviation characteristics of these genes do not allow us to ascribe these changes directly to altered *FKH1* expression; however, the association of Fkh1 with all four CK2 subunits (Ho et al., 2002) suggests that its transcriptional activity might be affected by CK2.

CK2-linked G2/M phase genes

Genes that peak at G2/M phase form the second largest group of altered genes. Transcription of 54 genes was affected, with repression and elevation occurring equally frequently. Again, chromatin-structure-linked genes, such as *SRI1* (repressed), whose product interacts with the Swi/SNF and RSC remodeling complex, and *AHC1* (elevated), encoding a Ada histone acetyltransferase complex component, exhibited expression deviations. Remarkably, transcript levels of the G1 cyclin Cln3, whose cooperation with Cdc28 (also named Cdk1) triggers the first step of a new cell cycle in yeast, was repressed at the early time points in the catalytic subunit mutants, suggesting a possible cell cycle (re-)entry delay compared with the wildtype.

Spellman et al. defined a MCM cluster comprising 34 genes peaking at about the M/G1 boundary that is primarily involved in initiation of DNA replication (Spellman et al., 1998). Transcriptional levels of 15 MCM cluster genes classified as G2/M specific were altered in one or more of the CK2 mutants. Two interesting MCM cluster genes exhibiting significantly different deviation characteristics between the regulatory subunit double mutant and the catalytic subunit deletion strains are represented by *SPO12* and *TSM1*, which encode a putative positive regulator of mitosis and TAF150 (see Fig. 1), respectively. *TSM1* expression was significantly elevated at all time points in the catalytic subunit mutants (except *cka1*Δ at 0 minutes) but only showed an elevation in the *ckb1*Δ *ckb2*Δ strain at 7 minutes. Conversely, *SPO12* was found continuously repressed in the regulatory subunit double mutant but virtually unaltered in the catalytic subunit deletion strains. Most MCM cluster genes contain binding sites for the transcription factor Mcm1 in their promoter regions (Spellman et al., 1998). We detected expression deviations at cell cycle (re-)entry for 10 out of the 27 genes found inside and outside of the MCM cluster identified as Mcm1-regulated by Simon et al. (Simon et al., 2001). Mcm1 is an essential, multifunctional cell cycle regulator that is highly homologous to human serum response factor (Kuo et al., 1997). Differential phosphorylation of Mcm1 may modulate its transcriptional activity on specific cofactors. Kuo et al. have identified more than eight differently phosphorylated Mcm1 isoforms (Kuo et al., 1997), including one that is necessary for the osmotic stress response. There is strong evidence that this specific Mcm1 phosphorylation is provided by protein kinase CK2, which has also been demonstrated to be involved in salt tolerance pathways (Glover, 1998). It is conceivable that CK2-mediated phosphorylation of Mcm1 – possibly at additional sites – causes expression deviations of Mcm1-regulated genes related to further physiological and cell cycle functions.

CK2-linked M/G1 phase gene

Transcription levels of 26 M/G1-specific genes were significantly affected by CK2 perturbation at cell cycle entry. Generally, repression was dominant (it was observed for two thirds of the altered M/G1 genes) and was most prominent in the regulatory subunit deletion strain. Several primarily repressed genes are associated with metabolic pathways and nutritional supply. Examples include the phosphate transporter gene *PHO89* and the long-chain fatty acid CoA-ligase gene *FAA1*, which exhibit various degrees of repression in the three CK2 mutants. The gene products of *FAA1*, encoded on chromosome 15, and its putative duplication on chromosome 8, *FAA4*, which, by contrast, showed elevated expression in the catalytic CK2 subunit mutants, are involved in fatty acid import (Faergeman et al., 2001). Remarkably, *YGP1*, which encodes a glycoprotein synthesized in response to starvation conditions, exhibited elevated transcription levels in the *ckb1*Δ *ckb2*Δ and *cka2*Δ strains, suggesting an impaired nutrient supply owing to CK2 perturbation.

Six of the altered M/G1 genes belong to a SIC1 cluster, which comprises 27 genes peaking in late M or at the M/G1 boundary (Spellman et al., 1998). There was no common expressional deviation pattern for these genes; instead, characteristics were quite divergent with respect to the direction of alteration (repression or elevation) and to the CK2

mutants concerned. A further four SIC1 cluster genes (*CTS1, YER124C, YHR143W* and *SCW11*) affected were classified by Spellman et al. as G1 specific (see above) (Spellman et al., 1998). Similarly, two altered MCM cluster genes (*HST4* and *YGP1*) have been characterized as M/G1 specific (Spellman et al., 1998). The fact that temporally coregulated genes are categorized into the same cluster but assigned to different cell cycle stages demonstrates that phasing of cell-cycle-regulated genes can only be crude (Spellman et al., 1998) and includes many borderline cases.

CK2-linked genes without phase categorization

Another three cell-cycle-regulated genes that peak at multiple phases (Cho et al., 1998) exhibited significant deviations in expression. Among these genes whose transcript levels were primarily altered in the regulatory subunit double mutant were the DNA helicase gene *RRM3* (repressed) and *BUD2* (elevated), which encodes a GTPase-activating protein involved in bud site selection that is necessary for viability in the absence of the G1 cyclin Cln3 (Benton et al., 1993).

Discussion

We carried out our search for the global roles of protein kinase CK2 using genome-wide expression profiling as this type of analysis provides the necessary global data. It allows a comprehensive comparison of genes whose expression levels are affected by perturbation of CK2 and discloses specific effects of the individual CK2 subunits. Genome-wide analysis was expected to reveal whether CK2 acts through defined transcription control features common to diverse genes or through higher-order structural processes such as chromatin remodeling. In the latter case, protein kinase CK2 would act as a global (unspecific) effector of gene expression. We present here for the first time a comprehensive dataset addressing this issue (Tables 1 and 2).

Our analysis shows that the expression levels of a relatively high proportion of genes, roughly a quarter of the 900 estimated to be cell-cycle-regulated in yeast (Spellman et al., 1998; Cho et al., 1998), are affected by protein kinase CK2 and are related to cell cycle (re-)entry from a G0-like state (pheromone arrest). In addition, 58 genes are also altered in permanently cycling cells (asynchronous cultures), and these represent rather persistent perturbation effects. According to the expression peaks, both groups comprise genes from various cell cycle phases. While not surprising for permanently cycling cells, it is quite remarkable for cells (re-)entering the cell cycle as these cells were expected to depend on early genes. Genes with persistently altered expression are primarily involved in metabolic pathways, nutritional supply and the pheromone response. This group, therefore, is not specific for cell cycle entry per se. These genes have various expression features in common. For instance, all significantly affected MET genes, members of a gene cluster responsible for methionine synthesis, exhibit the same CK2 subunit- and isoform-specific expression alteration, that is, repression in *ckb1*Δ *ckb2*Δ and *cka2*Δ strains but elevation in the *cka1*Δ strain. Similarly, transcription of the *PHO* genes, a gene cluster essential for phosphate supply, is strongly repressed in the *ckb1* Δ *ckb2* Δ strain, and the same is true for their common transcriptional activator Pho4. As this activator is subject to the control of a cell-cycle-linked regulatory complex whose components are CK2-independently expressed, CK2 perturbation uncouples *PHO* gene expression from its regulator (Barz et al., 2003). It should be noted that as phosphate supply is vital for all cells, this observation supports the survival factor hypothesis of CK2 (Ahmed et al., 2002).

The most interesting genes related to cell cycle (re-)entry fall into two groups. The first group comprises, predominantly, genes linked to cell cycle progression and exit, including genes associated with cell cycle engine control as well as cell division and apoptotic machineries (Fig. 5). The genes linked to cell cycle engine control include cyclin-encoding genes and genes encoding cyclin destruction proteins. For instance, *CLN3*, which encodes the cell cycle start cyclin in *S. cerevisiae*, is

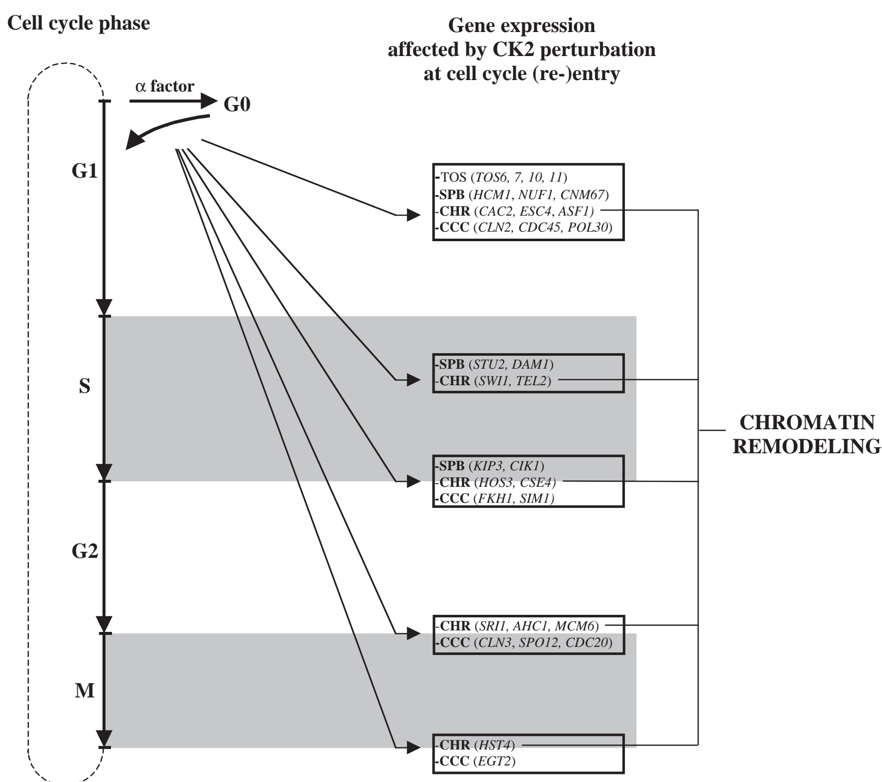


Fig. 5. Effects of CK2 perturbation at cell cycle entry on expression of cell-cycle-regulated genes. When (re-)entering the cell cycle following α factor arrest (a differentiated, G0-like state), CK2 mutants show significant expression deviations for genes specific for all cell cycle stages. Affected genes are positioned in boxes according to their phase-specific peak expression. Genes are functionally linked to the SPB, TOS (topoisomerase 2), CHR, (chromatin remodeling) and CCC (cell cycle control).

repressed in *cka*Δ strains, indicating a requirement for CK2 phosphorylation function for expression. *CLN2*, which encodes a cyclin active at G1/S transition and is induced by the Cln3-avtivated central yeast CDK Cdc28, is repressed in *ckb*Δ strains, indicating a requirement for the regulatory CK2 subunits. Further, we find a Cka1-linked increase in expression in *CDC20*, which encodes a subunit of the anaphase-promoting complex, which is involved in proteolysis of M phase cyclins. Another exit-related gene is *SPO12*, which encodes a putative positive exit regulator that requires Ckb1/Ckb2 for expression. Prominent among the genes related to the cell division machinery are genes encoding SPB components and SPB interaction partners. These include *HCM1*, which encodes a transcription factor involved in the regulation of SPB assembly, the SPB component gene *NUF1,* and *CIK1*, which encodes another SPB-associated protein. Remarkably, these SPB genes exhibit high CK2-subunit- and isoform-specific expression dependencies.

The second group comprises genes with a striking link to chromatin remodeling and modification. Chromatin-modifying complexes are divided into two main groups: ATP-dependent chromatin-remodeling complexes that use energy for nucleosome sliding along the DNA fibre and thus disrupt histone-DNA contacts (Gasser, 2002; Becker, 2002); and histone-modifying complexes, which add or remove covalent modifications from histone tails that influence histone-DNA binding and thus affect gene activation and silencing (Cosma, 2002). An exactly coordinated cooperation between chromatin-remodeling complexes, histone modifyers, general transcription factors and specific activators is required for successful transcription of a certain gene. For each cell cycle phase, we find altered expression of genes encoding proteins involved in this important process (Fig. 6). They are involved in silencing mechanisms (*ESC4* and *TEL2*) and nucleosome assembly (*ASF1, CAC2* and *CSE4*) or represent chromatin-remodeling components (*SWI1* and *SRI1*), a DNA helicase (*MCM6*) and histone (de)acetylases (*HOS3, AHC1, HST4*). In common with the SPB genes, their expression dependence on different CK2 subunits and isoforms is highly heterogenous, and – in contrast to the persistent alterations – shared deviation patterns for functionally related and/or commonly regulated genes (e.g. by identical transcription factors) could not be detected. Conversely, our promoter analyses of genes assigned to one cluster according to similar deviation characteristics did not reveal any potential upstream regulatory sequences that might indicate shared transcriptional regulation (data not shown). The indicated participation of CK2 in chromatin remodeling might explain this apparently ‘undirected’ effect of CK2 perturbation at cell cycle (re-)entry.

In addition to the indicated involvement of CK2 in the expression of chromatin-remodeling genes at cell cycle (re-)entry, evidence for CK2 implications in remodeling processes at the protein level is increasing. In yeast, all CK2 subunits have been discovered in complex with histones and general chromosomal remodeling factors (Gavin et al., 2002; Ho et al., 2002; Krogan et al., 2002). Phosphorylation of the nucleosome assembly proteins NAP-1 and -2 by CK2 is supposed to control nuclear-cytoplasmic histone translocation in fly and man, respectively (Li et al., 1999; Rodriguez et al., 2000). Furthermore, architectural non-histone chromatin components, such as the high mobility group proteins (HMGs) and the heterochromatin-associated protein 1 (HP1), are phosphorylated by CK2, affecting their DNA binding and gene silencing activities as well as their interaction with specific transcription factors (Wisniewski et al., 1999; Zhao et al., 2001; Krohn et al., 2002). It has also been shown that CK2 interacts with the basic leucine-zipper domains of several transcription factors such as ATF1, CREB, c-Fos or c-Jun, and in this way indirectly binds to DNA so that it can phosphorylate not only its immediate interacting partner but also proteins bound to nearby elements (Yamaguchi et al., 1998). The yeast ATF1/CREB homologue Sko1 regulates transcription of hyperosmotic-stress-induced genes such as the sodium pump gene *ENA1*, whose expression is defective in *ckb*Δ strains (Glover, 1998). Interestingly, Sko1 is important for recruitment of SWI/SNF nucleosome remodeling complexes and SAGA histone acetylase to osmotic-inducible promoters (Proft and Struhl, 2002). CK2-mediated phosphorylation of human histone deacetylases 1 and 2 (HDAC1/2) has been reported to promote complex formation and enzymatic activity (Pflum et al., 2001; Tsai and Seto, 2002; Sun et al., 2002). Moreover, Guo et al. found that CK2-nucleosome association and transcription initiation coincide at previously inactive chromatin regions, suggesting that CK2 participates in promoting the transition to a transcriptionally active conformation (Guo et al., 1998). Consistent with this, evidence for a differential CK2-mediated phosphorylation of nucleosomal proteins depending on the state of transcriptional activity has been presented (Guo et al., 1999).

Collectively the data strongly suggest that, independent from CK2 involvement in the regulation of specific gene groups (such as the PHO or MET genes), there is a global role for protein kinase CK2 in gene expression, which is based on its functions in essential chromatin-remodeling processes. If this was the case, a perturbation of CK2 would have a general, far-reaching and partly undirected effect on gene expression that is particularly pronounced at transition points such as cell cycle (re-)entry, where switching from one gene expression program to another requires significant chromatin reorganization. That this effect is particularly reflected by altered transcript levels of remodeling-associated genes might be a simple result of their preferred induction at cell cycle entry where their gene products are particularly needed.

We thank C. V. C. Glover for generously providing the yeast strains and critical reading of the manuscript. We also thank M. Hergenhahn for assistance in the Affymetrix GeneChip system handling, Kurt Fellenberg and Christian Busold for help with M-CHIPS and the members of the Biochemical Cell Physiology group for discussion. The work was supported by the Strategiefonds III Program of the Helmholtz Gesellschaft Deutscher Forschungszentren. This article is dedicated to Harald zur Hausen on occasion of his retirement as head of the Deutsches Krebsforschungszentrum with appreciation for two decades of leadership.

References

Ackermann, K., Waxmann, A., Glover, C. V. C. and Pyerin, W. (2001). Genes targeted by CK2: A genome-wide expression array analysis in yeast. *Mol. Cell. Biochem.* **227**, 59-66.

Ahmed, K., Gerber, D. A. and Cochet, C. (2002). Joining the cell survival squad: an emerging role for protein kinase CK2. *Trends Cell. Biol.* **12**, 226-230.

Alghisi, G. C., Roberts, E., Cardenas, M. E. and Gasser, S. M. (1994). The

regulation of DNA topoisomerase II by casein kinase II. *Cell. Mol. Biol. Res.* **40**, 563-571.

Alter, O., Brown, P. O. and Botstein, D. (2000). Singular value decomposition for genome-wide expression data processing and modeling. *Proc. Natl. Acad. Sci. USA* **97**, 10101-10106.

Barz, T., Ackermann, K. and Pyerin, W. (2003). Pertubation of protein kinase CK2 uncouples executive part of phosphate maintenance pathway from cyclin-CDK control. *FEBS Lett.* **537**, 210-214.

Becker, P. B. (2002). Nucleosome sliding: facts and fiction. *EMBO J.* **21**, 4749-4753.

Beissbarth, T., Fellenberg, K., Brors, B., Arribas-Prat, R., Boer, J. M., Hauser, N. C., Scheideler, M., Hoheisel, J. D., Schütz, G. et al. (2000). Processing and quality control of DNA array hybridization data. *Bioinformatics* **16**, 1014-1022.

Benton, B. K., Tinkelenberg, A. H., Jean, D., Plump, S. D. and Cross, F. R. (1993). Genetic analysis of Cln/Cdc28 regulation of cell morphogenesis in budding yeast. *EMBO J.* **12**, 5267-5275.

Bidwai, A. P., Reed, J. C. and Glover, C. V. C. (1995). Cloning and disruption of CKB1, the gene encoding the 38-kDa β subunit of *Saccharomyces cerevisiae* casein kinase II (CKII). *J. Biol. Chem.* **270**, 10395-10414.

Bodenbach, L., Fauss, J., Robitzki, A., Krehan, A., Lorenz, P., Lozeman, F. J. and Pyerin, W. (1994). Recombinant human casein kinase II. A study with the complete set of subunits (alpha, alpha' and beta), site-directed autophosphorylation mutants and a bicistronically expressed holoenzyme. *Eur. J. Biochem.* **220**, 263-273.

Burnett, G. and Kennedy, E. P. (1954). The enzymatic phosphorylation of proteins. *J. Biol. Chem.* **211**, 969-980.

Chen-Wu, J. L., Padmanabha, R. and Glover, C. V. C. (1988). Isolation, sequencing, and disruption of the CKA1 gene encoding the alpha subunit of yeast casein kinase II. *Mol. Cell. Biol.* **8**, 4981-4990.

Cho, J. R., Campbell, M. J., Winzeler, E. A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T. L., Gabrielian, A. T., Landsman, D. et al. (1998). A genome-wide transcriptional analysis of the mitotic cell cycle. *Mol. Cell* **2**, 65-73.

Cosma, M. P. (2002). Ordered recruitment: gene-specific mechanism of transcription activation. *Mol. Cell* **10**, 227-236.

DeRisi, J. L., Iyer, V. R. and Brown, P. O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680-686.

Dotan, I., Ziv, E., Dafni, N., Beckman, J. S., McCann, R. O., Glover, C. V. C. and Canaani, D. (2001). Functional conservation between human, nematode and yeast CK2 cell cycle genes. *Biochem. Biophys. Res. Commun.* **288**, 603-609.

Eden, A. and Benvenisty, N. (1999). Involvement of branched-chain amino acid aminotransferase (Bcat1/Eca39) in apoptosis. *FEBS Lett.* **457**, 255-261.

Faergeman, N. J., Black, P. N., Zhao, X. D., Knudsen, J. and DiRusso, C. C. (2001). The acyl-CoA synthetases encoded within FAA1 and FAA4 in *Saccharomyces cerevisiae* function as components of the fatty acid transport system linking import, activation, and intracellular utilization. *J. Biol. Chem.* **276**, 37051-37059.

Fellenberg, K., Hauser, N. C., Brors, B., Neutzner, A., Hoheisel, J. D. and Vingron, M. (2001). Correspondence analysis applied to microarray data. *Proc. Natl. Acad. Sci. USA* **98**, 10781-10786.

Fellenberg, K., Hauser, N. C., Brors, B., Hoheisel, J. D. and Vingron, M. (2002). Microarray data warehouse allowing for inclusion of experiment annotations in statistical analysis. *Bioinformatics* **18**, 423-433.

Gasser, S. M. (2002). Visualizing chromatin dynamics in interphase nuclei. *Science* **296**, 1412-1416.

Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A. M. et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141-147.

Glover, C. V. C. (1998). On the physiological role of casein kinase II in *Saccharomyces cerevisiae*. *Prog. Nucleic Acids Res.* **59**, 95-133.

Guo, C., Davis, A. T. and Ahmed, K. (1998). Dynamics of protein kinase CK2 association with nucleosomes in relation to transcriptional activity. *J. Biol. Chem.* **273**, 13675-13680.

Guo, C., Davis, A. T., Yu, S., Tawfic, S. and Ahmed, K. (1999). Role of protein kinase CK2 in phosphorylation of nucleosomal proteins in relation to transcriptional activity. *Mol. Cell. Biochem.* **191**, 135-142.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G. D., Moore, L., Adams, S. L., Millar, A., Taylor, P., Bennett, K. et al. (2002). Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. *Nature* **415**, 180-183.

Iyer, V. R., Eisen, M. B., Ross, D. T., Schuler, G., Moore, T., Lee, J. C., Trent, J. M., Staudt, L. M., Hudson, J. Jr et al. (1999). The transcriptional program in the response of human fibroblasts to serum. *Science* **283**, 83-87.

Krogan, N. J., Kim, M., Ahn, S. H., Zhong, G., Kobor, M. S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S. and Greenblatt, J. F. (2002). RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. *Mol. Cell. Biol.* **22**, 6979-6992.

Krohn, N. M., Yanagisawa, S. and Grasser, K. D. (2002). Specificity of the stimulatory interaction between chromosomal HMGB proteins and the transcription factor Dof2 and its negative regulation by protein kinase CK2-mediated phosphorylation. *J. Biol. Chem.* **277**, 32438-32444.

Kuo, M.-H., Nadeau, E. T. and Grayhack, E. J. (1997). Multiple phosphorylated forms of the Saccharomyces cerevisiae Mcm1 protein include an isoform induced in response to high salt concentrations. *Mol. Cell. Biol.* **17**, 819-832.

Landesmann-Bollag, E., Romieu-Mourez, R., Song, H. D., Sonenshein, G. E., Cardiff, R. D. and Seldin, D. C. (2001). Protein kinase CK2 in mammary gland tumorigenesis. *Oncogene* **20**, 3247-3257.

Li, M., Strand, D., Krehan, A., Pyerin, W., Heid, H., Neumann, B. and Mechler, B. M. (1999). Casein kinase 2 binds and phosphorylates the nucleosome assembly protein-1 (NAP1) in *Drosophila melanogaster*. *J. Mol. Biol.* **293**, 1067-1084.

Li, P., Li, J., Muller, E., Otto, A., Dietz, R. and von Harsdorf, R. (2002). Phosphorylation by protein kinase CK2. A signaling switch for the caspase-inhibiting protein ARC. *Mol. Cell.* **10**, 247-258.

Lorenz, P., Pepperkok, R., Ansorge, W. and Pyerin, W. (1993). Cell biological studies with monoclonal and polyclonal antibodies against human casein kinase II subunit beta demonstrate participation of the kinase in mitogenic signaling. *J. Biol. Chem.* **268**, 2733-2739.

Lorenz, P., Ackermann, K., Simoes-Wuest, P. and Pyerin, W. (1999). Serum-stimulated cell cycle entry of fibroblasts requires undisturbed phosphorylation and non-phosphorylation interactions of the catalytic subunits of protein kinase CK2. *FEBS Lett.* **448**, 283-288.

Lycan, D., Mikesell, G., Bunger, M. and Breeden, L. (1994). Differential effects of Cdc68 on cell cycle-regulated promoters in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14**, 7455-7465.

Nash, R., Tokiwa, G., Anand, S., Erickson, K. and Futcher, A. B. (1988). The whi1+ gene of Saccharomyces cerevisiae tethers cell division to cell size and is a cyclin homolog. *EMBO J.* **7**, 4335-4346.

Niefind, K., Guerra, B., Ermakowa, I. and Issinger, O. G. (2001). Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme. *EMBO J.* **20**, 5320-5331.

ole-MoiYoi, O. K. (1995). Casein kinase II in theileriosis. *Science* **267**, 834-836.

Padmanabha, R., Chen-Wu, J. L., Hanna, D. E. and Glover, C. V. C. (1990). Isolation, sequencing, and disruption of the yeast CKA2 gene: casein kinase II is essential for viability in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**, 4089-4099.

Pepperkok, R., Herr, S., Lorenz, P., Pyerin, W. and Ansorge, W. (1993). System for quantitation of gene expression in single cells by computerized microimaging: application to c-fos expression after microinjection of anti-casein kinase II antibody. *Exp. Cell Res.* **204**, 278-285.

Pepperkok, R., Lorenz, P., Ansorge, W. and Pyerin, W. (1994). Casein kinase II is required for transition of G0/G1, early G1, and G1/S phases of the cell cycle. *J. Biol. Chem.* **269**, 6986-6991.

Pflum, M. K., Tong, J. K, Lane, W. S. and Schreiber, S. L. (2001). Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation. *J. Biol. Chem.* **276**, 47733-47741.

Pinna, L. A. (2002). Protein kinase CK2: a challenge to canons. *J. Cell. Sci.* **115**, 3873-3878.

Proft, M. and Struhl, K. (2002). Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. *Mol. Cell* **9**, 1307-1317.

Rodriguez, P., Pelletier, J., Price, G. B. and Zannis-Hadjopoulos, M. (2000). NAP-2: histone chaperone function and phosphorylation state through the cell cycle. *J. Mol. Biol.* **298**, 225-238.

Schuldiner, O., Eden, A., Ben-Yosef, T., Yanuka, O., Simchen, G. and Benvenisty, N. (1996). ECA39, a conserved gene regulated by c-Myc in mice, is involved in G1/S cell cycle regulation in yeast. *Proc. Natl. Acad. Sci. USA* **93**, 7143-7148.

Seldin, D. C. and Leder, P. (1995). Casein kinase II alpha transgene-induced murine lymphoma: relation to theileriosis in cattle. *Science* **267**, 894-897.

Simon, I., Barnett, J., Hannett, N., Harbison, C. T., Rinaldi, N. J., Volkert,

T. L., Wyrick, J. L., Zeitlinger, J., Gifford, D. K. et al. (2001). Serial regulation of transcriptional regulators in the yeast cell cycle. *Cell* **106**, 697-708.

Spellman, P. T., Sherlock, G., Zhang, M. Q., Iyer, V. R., Anders, K., Eisen, M. B., Brown, P. O., Botstein, D. and Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell* **9**, 3273-3297.

Sun, J. M., Chen, H. Y., Moniwa, M., Litchfield, D. W., Seto, E. and Davie, J. R. (2002). The transcriptional repressor Sp3 is associated with CK2-phosphorylated histone deacetylase 2. *J. Biol. Chem.* **277**, 35783-35786.

Thomas, W., Spell, R. M., Ming, M. E. and Holm, C. (1991). Genetic analysis of the gyrase A-like domain of DNA topoisomerase II of *Saccharomyces cerevisiae*. *Genetics* **128**, 703-716.

Tsai, S. C. and Seto, E. (2002). Regulation of histone deacetylase 2 by protein kinase CK2. *J. Biol. Chem.* **277**, 31826-31833.

Vilk, G., Saulnier, R. B., St Pierre, R. and Litchfield, D. W. (1999). Inducible expression of protein kinase CK2 in mammalian cells. Evidence for functional specialization of CK2 isoforms. *J. Biol. Chem.* **274**, 14406-14414.

Wisniewski, J. R., Szewczuk, Z., Petry, I., Schwanbeck, R. and Renner, U. (1999). Constitutive phosphorylation of the acidic tails of the high mobility group 1 proteins by casein kinase II alters their conformation, stability, and DNA binding specificity. *J. Biol. Chem.* **274**, 20116-20122.

Wittmeyer, J. and Formosa, T. (1997). The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol. Cell. Biol.* **17**, 4178-4190.

Yamaguchi, Y., Wada, T., Suzuki, F., Takagi, T., Hasegawa, J. and Handa, H. (1998). Casein kinase II interacts with the bZIP domains of several transcription factors. *Nucleic Acids Res.* **26**, 3854-3861.

Yamaki, M., Umehara, T., Chimura, T. and Horikoshi, M. (2001). Cell death with predominant apoptotic features in *Saccharomyces cerevisiae* mediated by deletion of the histone chaperone ASF1/CIA1. *Genes Cells* **6**, 1043-1054.

Zhao, T., Heyduk, T. and Eissenberg, J. C. (2001). Phosphorylation site mutations in heterochromatin protein 1 (HP1) reduce or eliminate silencing activity. *J. Biol. Chem.* **276**, 9512-9518.