



## Yeast Functional Analysis Report

# Localization of proteins that are coordinately expressed with Cln2 during the cell cycle

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## Abstract

The localization of proteins can give important clues about their function and help sort data from large-scale proteomic screens. Forty-five proteins were tagged with the GFP variant YFP. These proteins were chosen because they are encoded by genes that display strong cell cycle-dependent expression that peaks in G<sub>1</sub>. Most of these proteins localize to either the nucleus or to sites of cell growth. We are able to assign new cellular component GO terms to ASF2, TOS4, RTT109, YBR070C, YKR090W, YOL007C, YOL019W and YPR174C. We also have localization data for 21 other proteins. Noteworthy localizations were found for Rfa1p, a member of the DNA replication A complex, and Pri2p and Pol12p, subunits of the  $\alpha$ -DNA polymerase : primase complex. In addition to its nuclear localization, Rfa1p assembled into cytoplasmic foci adjacent to the nucleus in cells during the G<sub>1</sub>–S phase transition of the cell cycle. Pri2 and Pol12 took on a beaded appearance at the G<sub>1</sub>–S transition and later in the cell cycle were enriched in the nuclear envelope. A new spindle pole body/nuclear envelope component encoded by YPR174 was identified. The cell cycle-dependent abundance of Tos4p mirrored Yox1p and these two proteins were the only proteins that were found exclusively at the G<sub>1</sub>–S phase of the cell cycle. A complete list of localizations, along with images, can be found at our website (<http://www.yeastrc.org/cln2/>). Copyright © 2004 John Wiley & Sons, Ltd.

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## Introduction

The sequencing of the yeast genome triggered a concerted effort to understand the function of every gene in the genome (Johnston, 2000). Large-scale genetic and biochemical approaches using a variety of techniques have been applied to extract relevant information on each gene. One approach, microarray analysis, has shown that >10% of all protein-coding genes are cell cycle-regulated (Cho *et al.*, 1998; Ihmels *et al.*, 2002; Spellman *et al.*, 1998; Zhao *et al.*, 2001). Genes with similar expression patterns often have similar functions (Eisen *et al.*, 1998; Spellman *et al.*, 1998), and recently several groups have used gene expression data to predict the function of a large number of genes (Li, 2002; Segal *et al.*, 2003; Wu *et al.*, 2002). However, it

is clear that gene expression profiles are not sufficient to assign function, e.g. 551 of the 656 genes differentially expressed during sporulation are not essential for high-efficiency sporulation or post-germination growth (Deutschbauer *et al.*, 2002).

The largest cluster of cell cycle-regulated genes that was reported by Spellman *et al.* (1998) was the 'CLN2' cluster. These genes peak in expression in mid-G<sub>1</sub> phase, are strongly induced by GAL–CLN3, and are strongly repressed by GAL–CLB2 (Spellman *et al.*, 1998). The genes in the CLN2 cluster with known functions are involved in processes such as DNA replication and cellular growth.

The localization of a protein often indicates the process that it performs because many cellular processes are limited spatially and temporally within

the cell. The discovery of green fluorescent protein (GFP) and its use as a fluorescent marker has made it possible to localize proteins in living cells (Niedenthal *et al.*, 1996; Tsien, 1998). By tagging a protein with GFP and maintaining the control of gene expression from the endogenous promoter, the localization of the tagged protein can be followed as it responds to changes in expression and other cellular cues. Thus, our goal was to evaluate the effectiveness of a targeted localization strategy to efficiently sort through proteins of interest identified by DNA microarray analysis.

## Materials and methods

### Media

The compositions of synthetic dextrose minimal medium (SD), yeast peptone dextrose rich broth medium (YPD) and selective media for transformants are described elsewhere (Burke *et al.*, 2000). SD complete (SDC) was SD with the addition of 0.2% casamino acids (Difco, Detroit, MI), 50 µg/ml adenine, 25 µg/ml uracil and 100 µg/ml tryptophan.

### Construction of strains containing YFP fusions

Genes of the CLN2 cluster were tagged with YFP using the PCR-based method described by Wach *et al.* (1997), using plasmid pDH6 (Hailey *et al.*, 2002) as described (Hazbun *et al.*, 2003). Strains were tagged in a diploid cell and haploids were obtained by either tetrad dissection or random sporulation (Burke *et al.*, 2000).

### Fluorescence microscopy

Cells carrying YFP fusion genes were grown on solid YPD medium supplemented with additional adenine overnight at 30 °C and then suspended in SDC medium. Cells were mounted in one of two ways. In the first method, an aliquot of cells (3 µl) was mixed with an equal volume of SDC containing 1.2% SeaPlaque low melting temperature agarose (FMC BioProducts), previously melted at 40 °C. The mixture was placed on a Gold Seal<sup>®</sup> microscope slide and a Corning No. 1½ cover slip was pressed firmly on top. Alternatively, a pad of 0.9% SeaKem LE agarose (FMC BioProducts) in SDC was created by placing 20–30 µl of the

melted agarose on a microscope slide that had two strips of Scotch Magic Tape taped across either end. A second slide was then laid down, sandwiching the agarose between the two slides. The agarose was allowed to solidify and the two slides were slid apart, leaving the agar pad on one of the slides. An aliquot of cells was placed on the pad and covered with a coverslip. The advantages of the second method were that the cells did not undergo heat shock, and all cells were in the same focal plane, ideally positioned adjacent to the coverslip.

Fluorescence microscopy was performed on a DeltaVision microscope with an Olympus IL-70 microscope, a CoolSnap digital camera from Roper Scientific and optical filter sets from Omega Optical. A single focal plane was captured and the raw image was exported in TIFF format to the database. TIFF format image files were exported from Softworx software and uploaded via a web interface into our online MySQL database. The images exist in the original binary form in the database. When the image is requested by a user on the web at <http://www.yeastrc.org/cln2/>, the image manipulation library ImageMagick converts the raw TIFF data into a PNG image suitable for display in a web browser. Information from the microscopy log files was parsed through a pattern recognition program that populated a database table associated with the captured image. Software code is available on request.

## Results and discussion

Genes that share an expression pattern with CLN2 were obtained from the data in Figure 3 of Spellman *et al.* (1998) and from a search for 80 genes that are expressed similarly to CLN2 at Stanford's Cell Cycle website (<http://cellcycle-www.stanford.edu/>). From this list of 96 genes, 45 were chosen and tagged with the GFP variant YFP. The 45 were chosen with an emphasis on uncharacterized ORFs and avoiding ORFs considered questionable by the Munich Information Centre for Protein Sequences (MIPS). We used YFP to facilitate later dual labelling with another GFP variant, CFP.

From the analysis of Spellman *et al.* (1998), all 45 genes are expressed in a periodic manner with a peak in the mid- to late G<sub>1</sub> phase of

the cell cycle. Two other statistical approaches have also concluded that most of these genes are expressed periodically (Table 1). A statistical modelling approach found that expression of 34 of the 45 genes oscillated with high confidence values, with 28 genes coordinate with *Cln2* (Zhao *et al.*, 2001). Using a non-clustering algorithm, 31 of the 45 genes were assigned to the  $G_1$  transcriptional module (Ihmels *et al.*, 2002). Thus, there is a general consensus that the set of 45 genes display a periodic transcription that peaks in  $G_1$ .

Periodic transcription does not *a priori* imply periodic protein abundance. Stable proteins may require a brief period of increased transcription to satisfy a demand for a protein during a window of assembly. Thus, in order to secure an ample supply of proteins that constitute chromatin or the spindle pole body, expression may increase before entry into S phase. On the other hand, a pulse of transcription followed by turnover of the transcript and protein would provide a simple mechanism to restrict the action of a protein to a specific time frame.

YFP was inserted prior to the stop codon of the endogenous gene, as described by Wach *et al.* (1997), such that the YFP-tagged gene remained under the control of the native promoter. A fluorescent signal was detected and localized for 29 of the 45 fusion proteins (Table 1). These localizations allowed the assignment of new cellular component GO terms to eight ORFs: ASF2, TOS4, RTT109, YBR070C, YKR090W, YOL007C, YOL019W and YPR174C. Eight fusion proteins showed striking localizations and are described in greater detail below. Twelve proteins were previously localized, either by immunofluorescence or as GFP fusion proteins. The complete set of localizations and images can be found at our website (<http://www.yeastrc.org/cln2/>).

Among the 29 detectable proteins, 14 were stable proteins that did not dramatically change localization during the cell cycle. The remaining 15 proteins had a diverse array of dynamic localizations that were contingent on the stage of the cell cycle (described in detail below with further images at <http://www.yeastrc.org/cln2/>). Yox1p–YFP and Tos4p–YFP appeared exclusively during the  $G_1$  and S phases of the cell cycle. Two subunits of the  $\alpha$ DNA polymerase:primase complex, Pri2p and Pol12p, moved to the nuclear periphery during nuclear division. Three proteins, the products

of YGR221c, YKR090w and SPH1, localized to the bud tip during  $G_1$  and also concentrated at the bud neck in late telophase. Ten gene products were detected at the bud neck, and for three, those of Rsr1p, Axl2p and Hsl1p, localization was detectable only in large-budded cells. If these proteins are part of a complex that is degraded during cytokinesis, then the increase of transcription in  $G_1$  may serve to replenish the proteins for the next budding cycle. These results highlight the myriad patterns of protein localizations that can emanate from a single set of coordinately regulated genes.

The eight proteins showing striking localizations include Yox1p and Tos4p, the two proteins restricted to  $G_1$  and S phases. Yox1p is a homeodomain transcription factor that acts as a transcriptional repressor at early cell cycle boxes (Pramila *et al.*, 2002). From Western blot analysis, protein levels of Yox1p peak at the  $G_1$ –S boundary (Pramila *et al.*, 2002). Consistent with those results, Yox1p–YFP fluorescence peaks in small-budded cells when fluorescence is four times brighter than other periods in the cell cycle (Figure 1A).

Tos4p is a transcription factor also involved in regulating gene transcription at the  $G_1$ –S boundary (Horak *et al.*, 2002). The nuclear fluorescence of Tos4p–YFP, like that of Yox1–YFP, peaked in large unbudded cells and very small-budded cells (Figure 1B). The results suggest that like Yox1p, a strict control of the level of the Tos4p will be part of the mechanism that restricts its activity to the  $G_1$ –S phase of the cell cycle.

Pri2p and Pol12p are subunits of the  $\alpha$ DNA polymerase:primase complex (Burgers, 1998) and, as expected, they share a similar localization pattern. Both proteins have a diffuse nuclear localization for most of the cell cycle, with some granularity appearing in small-budded cells. As the nucleus divides, the intensity of the signal in the interior of the nucleus weakens. The localization remains concentrated along the periphery of the nucleus, although this is subtle with Pol12p–YFP fusion (Figure 2). Thus, there is a dynamic change in the abundance and/or localization of the complex during S phase and mitosis.

Rfa1p is a member of the DNA replication A complex (Wold, 1997) and, as expected, Rfa1p–YFP was found in the nucleus throughout the cell cycle. However, in addition Rfa1p also concentrates in cytoplasmic foci (Figure 3A). When visualized, these patches were prominent in unbudded

**Table I.** In the column labelled  $G_1$ , A indicates a gene in the  $G_1$  module as defined in Ihmels et al. (2002); B, a gene with a cell cycle transcription pattern similar to CLN2; and C, a gene that displays periodic transcription but is distinct from CLN2 (Zhao et al., 2001). 'Cellular component' refers to the assigned GO term

Gene	ORF	$G_1$	Cellular component	Description of localization
SWD1	YAR003W		Nucleus	Throughout nucleus
RFA1	YAR007C	A, B	Nucleus, cytoplasm	Throughout nucleus, punctate dots in cytoplasm typically adjacent to nucleus in unbudded or small-budded cells
POL12	YBL035C	A, B	Nucleus	Just prior to nuclear division the signal is brightest and is throughout nucleus with some punctate patterning. As nucleus divides it is more noticeably at the nuclear periphery
	YBR070C	A, B	Nuclear envelope—endoplasmic reticulum network	Mottled network surrounding nucleus and extending throughout cell
	YBR071W	A, C	Unknown	No signal detected
POL30	YBR088C	A, B	Nucleus	Throughout nucleus with occasional punctate patterning Cells are large and unhealthy
KCC4	YCL024W	A, B	Bud neck	Daughter side of bud neck, forms ring at incipient bud site, weak signal in large-budded cells
MCD1	YDL003W	A, B	Nucleus	Only in medium to large-budded cells
	YDL010W		Unknown	No signal detected
ASF2	YDL197C	A, C	Telomeric chromatin	Punctate along nuclear periphery
GIC2	YDR309C		Unknown	No signal detected
CWH41	YGL027C		Unknown	No signal detected
RSR1	YGR152C	A, B	Bud tip, bud neck	Daughter side of bud neck
	YGR221C	A, B	Bud tip, bud neck	Punctate at bud tip, weak signal at bud neck in large-budded cells
SPO16	YHR153C	A, B	Unknown	No signal detected
AXL2	YIL140W	A, C	Bud tip, bud neck	Daughter side of bud neck, localization diffuses out toward bud tip and sometimes toward the mother
MPS3	YJL019W	B	Spindle pole body	Classic spindle pole localization
PSF2	YJL072C		Nucleus	Throughout nucleus
JEM1	YJL073W		Nuclear envelope—endoplasmic reticulum network	Diffuse throughout cell, with a tie-dyed patterning that is centred around nucleus
SMC3	YJL074C	A, B	Nucleus	Throughout nucleus, occasional beaded pattern
	YJL181W	A, B	Unknown	No signal detected
SWE1	YJL187C	A, B	Bud neck, nucleus	Daughter side of bud neck
PRI2	YKL045W	A, B	Nucleus	Bright and primarily diffuse in the nucleus. Occasional beaded nuclear pattern in small-budded cells. As nucleus divides noticeably enriched along nuclear periphery
HSL1	YKL101W	A, B	Bud neck	Daughter side of bud neck, not seen in all large-budded cells
	YKR090W	A	Bud tip, bud neck	Diffuse at bud tip, at the tip in large-budded cells
RTT109	YLL002W	A, B	Nucleus	Diffuse throughout the nucleus
TOS4	YLR183C	A, B	Nucleus	Seen in nucleus only in small to medium-budded cells
EST1	YLR233C		Unknown	No signal detected
SPH1	YLR313C	A, C	Bud tip, bud neck	Slightly punctate at the bud tip, at the bud neck in large-budded cells
YOX1	YML027W	A, B	Nucleus	Seen in nucleus only in small to medium-budded cells
CAC2	YML102W	B	Unknown	No signal detected
	YML133C		Unknown	No signal detected
CLN1	YMR199W	A, C	Unknown	No signal detected
BNI4	YNL233W	A, B	Bud neck	Mother side of bud neck
	YOL007C	A, B	Bud neck, vacuole	Mother side of bud neck
ESC8	YOL017W	A, B	Unknown	No signal detected
	YOL019W		Plasma membrane	Punctate at cellular periphery, predominantly in the mother and not the bud
SLK19	YOR114W	A, C	Unknown	No signal detected
	YOR195W	B	Kinetochores, spindle mid-body	Classic dynamic kinetochore signal

Table I. Continued

Gene	ORF	G <sub>1</sub>	Cellular component	Description of localization
SVS1	YPL163C	A, B	Unknown	No signal detected
	YPL208W		Unknown	No signal detected
CLN2	YPL256C	A, B	Unknown	No signal detected
	YPL267W	A, B	Unknown	No signal detected
	YPRI74C	B	Nuclear membrane, spindle pole body	One or more bright spots on nuclear membrane that often correspond to the spindle pole body
DPB2	YPRI75W	A, B	Nucleus, cytoplasm	Strongest localization in nucleus, diffuse throughout cytoplasm

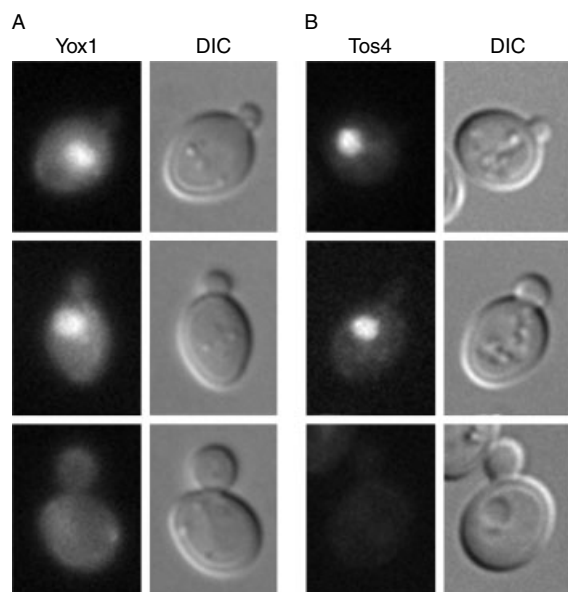


Figure 1. G<sub>1</sub>-specific localization. Yox1p and Tos4p were tagged with YFP and imaged as described in Material and methods. The fluorescent and corresponding DIC images are shown

(5/9), small-budded (1/9) or medium-budded (3/9) cells as a single dot, usually (8/9) adjacent to the nucleus. One or two brighter foci were sometimes seen within the nucleus. The timing of the appearance of the extranuclear localization suggests a role in an early stage of DNA replication, perhaps a processing stage of the protein before nuclear entry. Clearly this result warrants further investigation.

We should also note that most of the nuclear proteins had some perceptible structure in a subpopulation of cells that were examined (Figure 2; also see website). The nuclear signal was not always diffuse. The structures are best described as a subtle grains or beads. As an example, for Pri2p–YFP, 17 of the 22 cells with obvious granularity were small-budded cells, presumably at an early stage

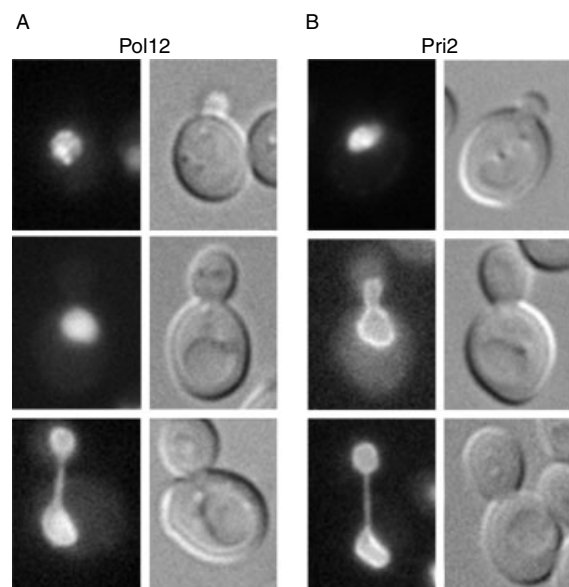
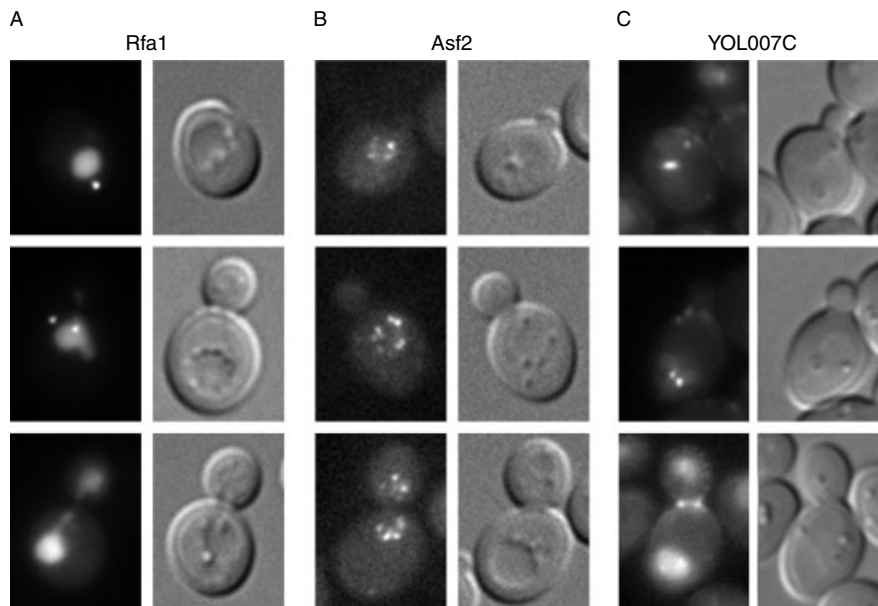


Figure 2. Localization of two subunits of the DNA polymerase:primase complex. Pol12p and Pri2p were tagged with YFP and imaged as described in Material and methods. The fluorescent and corresponding DIC images are shown

of DNA replication. It is tempting to speculate that the beaded fluorescence corresponds to foci of early replication analogous to the early replication foci seen in mammalian cells (Kennedy *et al.*, 2000).

Asf2p has an anti-silencing function at silent loci (Le *et al.*, 1997). Asf2p–YFP localizes to multiple foci within the nucleus similar to the speckled localization of telomeric proteins such as Rap1p (Figure 3B) (Laroche *et al.*, 2000). The telomeric localization of Asf2p suggests that Asf2p functions directly at the telomeres.

The localization of YOL007C–YFP is extraordinary because it was found at both a site of polarized growth and an organelle. YOL007C–YFP has a weak localization to the bud neck and a



**Figure 3.** Three novel localizations, Rfa1p, Asf2p and YOL007c, were tagged with YFP and imaged as described in Material and methods. The fluorescent and corresponding DIC images are shown

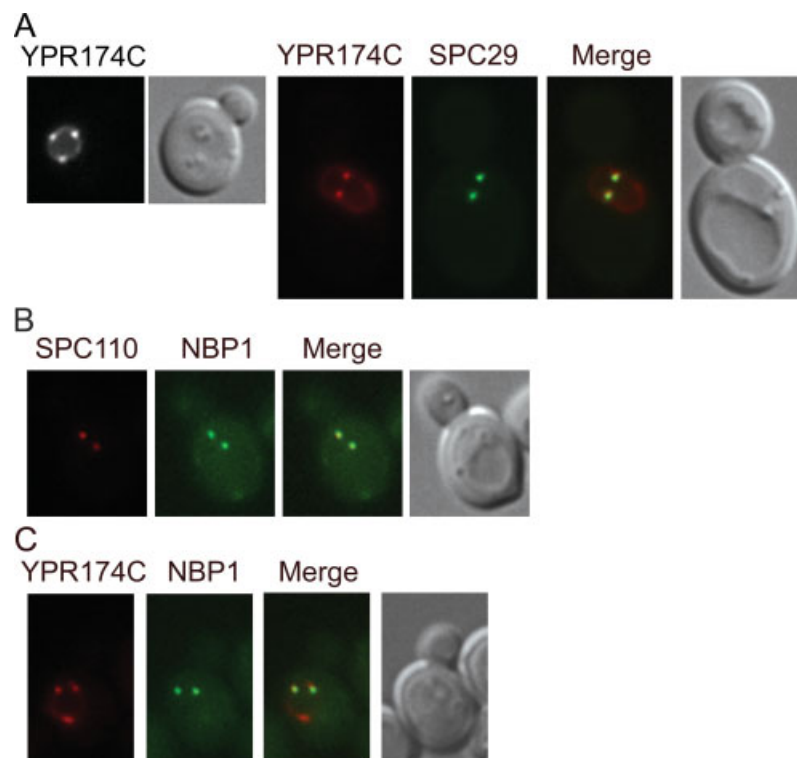
distinctive localization at the vacuole. There it is both dispersed throughout the vacuole and in individual foci (Figure 3C). The brightest foci were often found as one or two dots at the outer edge of the surface of the vacuole (7/10).

The protein YPR174c–YFP is located throughout the nuclear membrane (Figure 4). In one or more areas the localization is concentrated in bright foci. To investigate whether these foci were associated with the spindle pole body (SPB) their position was compared to the SPB component Spc29p–CFP. The foci of YPR174c–YFP predominantly (29/38) co-localized with the SPB (Figure 4A). Occasionally YPR174c–YFP was immediately adjacent to the SPB. The protein encoded by YPR174c has partial sequence similarity to Nbp1p, an essential protein of unknown function which was suggested to co-localize with the SPB (Shimizu *et al.*). We verified that Nbp1p co-localizes with the known SPB component Spc110p (51/51) and does not localize to any additional foci (Figure 4B). As predicted, YPR174c co-localizes with Nbp1 in a majority of cells (42/62) (Figure 4C). The localization of YPR174c suggests that YPR174c is a specialized component of the nuclear membrane that may be involved in the connection of the SPB to the nuclear envelope. It will be interesting to see whether YPR174c and Nbp1

interact with the Bbp1–Mps2 complex, which is involved in the insertion of the duplication plaque of the SPB into the nuclear envelope during SPB duplication (Schramm *et al.*, 2000).

During the course of this study, Huh *et al.* (2003) surveyed the localization of 4156 proteins of the yeast proteome. Huh *et al.* detected nine proteins (encoded by YBR071w, YDL010w, GIC2, CAC2, CLN1, ESC8, SVS1, YPL208w and CLN2) that we did not, and we detected five proteins (encoded by YBR070c, YGR221c, MPS3, SWE1, PRI2) that were not observed by Huh *et al.* The cause for this discrepancy awaits further investigation. None of our highlighted localizations (encoded by YOX1, TOS4, PRI2, POL12, RFA1, ASF2, YOL007c and YPR174c) were elaborated upon by Huh *et al.*, although some of their images hint at the localizations we describe. In cataloguing 75% of the proteomic landscape of yeast, the pioneering work of Huh *et al.* created an invaluable global map of protein localizations. The finer detail that arose from our focused study clarified the state of individual proteins.

In summary, we have used a targeted localization strategy to sort genes that were grouped together by their coordinate transcription. By several methods of analysis, these genes are preferentially transcribed in mid- to late G<sub>1</sub>. Most (17/29, 58%) of



**Figure 4.** Localization of new spindle pole body component. (A) YPR174c was tagged with YFP and imaged as described in Material and methods. The fluorescent and corresponding DIC images are shown. In addition, YPR174C–YFP was also imaged in a cell in which SPC29p was labelled with CFP. The YFP image was pseudo-coloured red and the CFP image was pseudo-coloured green. In the merged image, yellow represents the area of co-localization. (B) Spc110p–YFP and Nbp1p–CFP were co-localized with the same pseudo-colouring scheme as in A. (C) YPR174c–YFP was imaged in a cell in which NBP1 was labelled with CFP. The same pseudo-colouring scheme is the same as in (A)

our detectable YFP-tagged proteins localize to the nucleus or nuclear membrane. This is a two-fold enrichment over the proportion of nuclear proteins in the characterized fraction of the yeast genome. Of the 5772 ORFs in yeast (4114 verified and 1658 uncharacterized), 1282 ORFs have been assigned a GO-slim component term that places the gene product in the nucleus (Dolinski *et al.*, 25 September 2003). In the CLN2 cluster the greatest enrichment is in genes localized in the bud. Ten of our 29 proteins localize to the bud tip and/or neck, even though less than 2% of the yeast genome is annotated with the GO term 'bud'. Most (7/10) of these proteins are found exclusively at the bud neck in mid- to large-budded cells. Thus, for these proteins there is a long delay between increased transcription and the time in the cell cycle when they concentrate into a visible structure. Our study has identified several novel localizations that should provide a foundation for future research on the

structure of the spindle pole body and the DNA replication machinery.

In conclusion, protein localization is an effective tool to mine data on gene transcription obtained by microarray analysis. After all, gene expression culminates in the delivery of a protein to its place of action at a time when its function is required. By bringing together information on the timing of transcription with the acquisition of high-resolution images of the dynamics of protein localization, one spans the life of a protein. Thus, protein localization targeted to a subset of proteins linked by a common pattern of transcription promises to be an efficient and informative avenue to explore the proteome of any cell.

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## References

- Burgers PM. 1998. Eukaryotic DNA polymerases in DNA replication and DNA repair. *Chromosoma* **107**: 218–227.
- Burke D, Dawson D, Stearns T. 2000. *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press: New York.
- Cho RJ, Campbell MJ, Winzler EA. 1998. A genome-wide transcriptional analysis of the mitotic cell cycle. *Mol Cell* **2**: 65–73.
- Deutschbauer AM, Williams RM, Chu AM, Davis RW. 2002. Parallel phenotypic analysis of sporulation and postgermination growth in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **99**: 15 530–15 535.
- Dolinski K, Balakrishnan R, Christie KR, et al. *Saccharomyces Genome Database* (accessed 25 September 2003): [<http://www.yeastgenome.org>]
- Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* **95**: 14 863–14 868.
- Hailey DW, Davis TN, Muller EG. 2002. Fluorescence resonance energy transfer using colour variants of green fluorescent protein. *Methods Enzymol* **351**: 34–49.
- Hazbun TR, Malmstrom L, Anderson S, et al. 2003. Assigning function to yeast proteins by integration of technologies. *Mol Cell* **12**: 1353–1365.
- Horak CE, Luscombe NM, Qian J, et al. 2002. Complex transcriptional circuitry at the G<sub>1</sub>/S transition in *Saccharomyces cerevisiae*. *Genes Dev* **16**: 3017–3033.
- Huh WK, Falvo JV, Gerke LC, et al. 2003. Global analysis of protein localization in budding yeast. *Nature* **425**: 686–691.
- Ihmels J, Friedlander G, Bergmann S, et al. 2002. Revealing modular organization in the yeast transcriptional network. *Nature Genet* **31**: 370–377.
- Johnston M. 2000. The yeast genome: on the road to the Golden Age. *Curr Opin Genet Dev* **10**: 617–623.
- Kennedy BK, Barbie DA, Classon M, Dyson N, Harlow E. 2000. Nuclear organization of DNA replication in primary mammalian cells. *Genes Dev* **14**: 2855–2868.
- Laroche T, Martin SG, Tsai-Pflugfelder M, Gasser SM. 2000. The dynamics of yeast telomeres and silencing proteins through the cell cycle. *J Struct Biol* **129**: 159–174.
- Le S, Davis C, Konopka JB, Sternglanz R. 1997. Two new S-phase-specific genes from *Saccharomyces cerevisiae*. *Yeast* **13**: 1029–1042.
- Li KC. 2002. Genome-wide co-expression dynamics: theory and application. *Proc Natl Acad Sci USA* **99**: 16 875–16 880.
- Niedenthal RK, Riles L, Johnston M, Hegemann JH. 1996. Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. *Yeast* **12**: 773–786.
- Pramila T, Miles S, GuhaThakurta D, Jemioło D, Breeden LL. 2002. Conserved homeodomain proteins interact with MADS box protein Mcm1 to restrict ECB-dependent transcription to the M/G<sub>1</sub> phase of the cell cycle. *Genes Dev* **16**: 3034–3045.
- Schramm C, Elliott S, Shevchenko A, Schiebel E. 2000. The Bbp1p–Mps2p complex connects the SPB to the nuclear envelope and is essential for SPB duplication. *EMBO J* **19**: 421–433.
- Segal E, Battle A, Koller D. 2003. Decomposing gene expression into cellular processes. *Pac Symp Biocomput*: 89–100.
- Shimizu Y, Akashi T, Okuda A, Kikuchi A, Fukui K. 2000. NBP1 (Nap1 binding protein 1), an essential gene for G<sub>2</sub>/M transition of *Saccharomyces cerevisiae*, encodes a protein of distinct sub-nuclear localization. *Gene* **246**: 395–404.
- Spellman PT, Sherlock G, Zhang MQ, et al. 1998. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* **9**: 3273–3297.
- Tsien RY. 1998. The green fluorescent protein. *Annu Rev Biochem* **67**: 509–544.
- Wach A, Brachat A, Alberti-Segui C, Rebischung C, Philippsen P. 1997. Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast* **13**: 1065–1075.
- Wold MS. 1997. Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu Rev Biochem* **66**: 61–92.
- Wu LF, Hughes TR, Davierwala AP, et al. 2002. Large-scale prediction of *Saccharomyces cerevisiae* gene function using overlapping transcriptional clusters. *Nature Genet* **31**: 255–265.
- Zhao LP, Prentice R, Breeden L. 2001. Statistical modelling of large microarray data sets to identify stimulus–response profiles. *Proc Natl Acad Sci USA* **98**: 5631–5636.