The dynamics of cell wall biogenesis in yeast
Smits, G.J.

Citation for published version (APA):
Smits, G. J. (2000). The dynamics of cell wall biogenesis in yeast

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
TRANSCRIPTION OF MULTIPLE CELL WALL PROTEIN-ENCODING GENES IN SACCHAROMYCES CEREVISIAE IS DIFFERENTIALLY REGULATED DURING THE CELL CYCLE

L. Heleen P. Caro
Gertien J. Smits
Piet van Egmond
John W. Chapman
Frans M. Klis

This chapter was published, with minor modifications, in FEMS Microbiology Letters, 1998 Vol. 161: 345-349
The dynamics of cell wall biogenesis in yeast
ABSTRACT
The yeast cell wall consists of an internal skeletal layer and an outside protein layer. The synthesis of both β-1,3-glucan and chitin, which together form the cell wall skeleton, is cell cycle regulated. We show here that the expression of five cell wall protein-encoding genes (CWP1, CWP2, SED1, TIP1 and TIR1) is also cell cycle regulated. TIP1 is expressed in G1-phase, CWP1, CWP2 and TIR1 are expressed in S/G2-phase, and SED1 in M-phase. The data suggest that these proteins fulfill distinct functions in the cell wall.

INTRODUCTION
The cell wall of yeast may account for up to 30% of the dry weight of the cell, and therefore represents a major investment of the cell (Fleet 1991). The main components of the cell wall are mannoproteins and β-linked glucans, and a small amount of chitin (Cid et al. 1995, Klis 1994, Orlean 1997). The wall is responsible for the mechanical strength of yeast cells. As a result, the cell wall has often been envisioned as a static structure with a constant composition. Evidence is however accumulating that the cell wall may vary considerably in response to both external and internal cues.

For example, external cues such as heat- or hypo-osmotic shock, or changes in the carbon source, affect the transcription of several genes that encode cell wall biosynthetic enzymes, as well as several genes encoding glucanase-extractable cell wall proteins (Donzeau et al. 1996, Igual et al. 1996, Kamada et al. 1995, Kondo and Inouye 1991, Kowalski et al. 1995, Marguet and Lauquin 1986, Munoz-Dorado et al. 1994, Orlean 1997, Teunissen and Steensma 1995). Also, the biosynthesis of the glucan and chitin components of the cell wall is cell cycle regulated, both transcriptionally (Davenport et al. 1995, Igual et al. 1996, Pammer et al. 1992, Ram et al. 1995) and posttranslationally (see Orlean 1997).

We provide evidence that the transcription of five cell wall proteins is cell cycle regulated, and that these genes are expressed at different stages of the cell cycle. This suggests that these proteins, which have so far been regarded only as general structural cell wall proteins, have specific functions in cell wall construction.
The dynamics of cell wall biogenesis in yeast

MATERIALS AND METHODS

Yeast strains and growth conditions

Saccharomyces cerevisiae strain X2180-1A was obtained from the Yeast Genetic Stock Center, Berkeley, CA, USA. Cells were grown in YPD medium.

Synchronization

Synchronization by α-factor was performed basically according to de Nobel et al. (1991). For centrifugal elutriation, a 5-ml elutriator rotor JE-6B (Beckman Instruments BV, Mijdrecht, NL) was used. Cells were grown in batch cultures on YPD. Elutriation was performed as described by Woldringh et al. (1993). In both cases, samples were taken every 15 min. For microscopic analysis, cells were fixed with 0.13% (w/v) formaldehyde and sonicated briefly to break up aggregates. For RNA isolation, 20 ml of cell suspension were centrifuged, the cells were washed with ice-cold water, and stored in liquid nitrogen.

RNA-isolation and Northern hybridization

RNA was isolated by the hot-phenol method, without the use of glass beads, as described in Current Protocols (Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.). Ten μg of RNA per lane were separated on a formaldehyde/formamide containing agarose gel-system. RNA was cross-linked to Hybond-N+ membrane (Amersham) by UV radiation. Northern hybridizations were performed in the presence of 50% formamide at 42 °C using 32P-labeled gene fragments. The blots were washed at 42 °C with decreasing concentrations of SSC, down to 0.5XSSC, in the presence of 0.1% SDS.

Hybridization signals were quantified by scanning of autoradiograms in the linear range of the films. Expression levels were normalized to actin levels.

RESULTS

To study the cell cycle regulated expression of cell wall protein encoding genes, we synchronized growing cultures with α-factor and by centrifugal elutriation. The results were essentially the same, although with centrifugal elutriation less synchrony was achieved. When the cells are arrested in G1 with α-factor, it takes approximately one generation time (100 minutes at 23°C) until all
cells have reached G1. During this period the expression of the cell wall proteins changed (Figure 1): The mRNA levels of CWP1, CWP2, and TIR1 decreased, whereas those of SED1 and TIP1 increased. The increase in mRNA levels of SED1 and TIP1 indicates that the arrest by α-factor takes effect at a time when transcription of both genes is induced, whereas this is not the case for CWP1, CWP2, and TIR1 (see also Figure 2B). Alternatively, α-factor may have a direct effect on cell wall protein expression, but since the induction is slow, and no consensus sequences for binding of Ste12p are present in the promoter regions of SED1 and TIP1, the former possibility seems more likely.

The effect of G1 arrest on cell wall protein expression was corroborated when the transcription of the cell wall protein-encoding genes was followed during several division cycles in α-factor synchronized cultures (Figure 2A). The transcription of CWP1 peaked slightly later than H2A, at the time when most cells had small buds, which is estimated to be late in S-phase or early in G2. About 10 minutes later, the mRNA level of CWP2 reached its maximum, which coincided with the peak in the number of budded cells. It can therefore probably be placed in G2.
The dynamics of cell wall biogenesis in yeast

A

B

CLN2
H2A
CWP1
CWP2
TIR1
SED1
TIP1

FULL CYCLE
Although the peaks of CWP1 and CWP2 are slightly separated, the induction of transcription of these genes occurs around the same time, and might therefore be brought about by similar mechanisms. Approximately 30 minutes after CWP2, SED1 transcription peaked. Almost all visible buds were large buds at that time, indicating that this is M-phase. TIP1 transcription reached its maximum after SED1, but before CLN2. This seems to occur early in G1, around the time of cell separation, since the percentage of budded cells rapidly decreases at the time when TIP1 transcription was highest. The transcription of TIR1 was somewhat more diffuse, but we estimate TIR1 transcription to occur mainly in the same period as CWP1 and CWP2 (see Fig. 2B), based on the periodic increases in the mRNA level of this gene.

Assuming that mRNA levels start to increase when transcription is induced, and start to level or decrease (depending on the mRNA stability) when transcription ceases or is shifted down, we can determine the periods during which the genes are transcriptionally activated. A graphic representation of this is shown in Figure 2B, in which dark areas represent the periods of increased transcriptional activity.

**DISCUSSION**

Since yeast is a unicellular organism, it has to continuously respond to changes in the environment. The cell wall does not only function as a barrier between the cell and the environment, but is also the first contact with the outside. Therefore, it has to constantly adapt, to provide optimal protection from, and optimal interaction with the outside.

**Figure 2A:** Messenger RNA levels of various cell wall protein-encoding genes in α-factor synchronized cultures. All mRNA levels were normalized to ACT1 levels, and the highest value was set at 100%. The percentage of budded cells (■), small budded cells (□) and the mRNA levels of CLN2 and HTA1/2 (H2A) serve as cell cycle progression and synchronicity markers. Probes were used against CWP1, CWP2, TIR1, SED1, and TIP1 mRNA. Thin vertical lines indicate the times at which CLN2 mRNA is at its maximum. **B:** Transcriptionally active periods of cell wall protein-encoding genes. Depicted is a normalized cell cycle, which is the average of all three cycles from Fig. 2A. Averages of start and end of activated transcription of each gene were calculated relative to the peaks in CLN2 transcription.
Several of the five cell wall protein-encoding genes we have studied (out of a total of almost 40 (Caro et al. 1997)) are known to be activated under specific conditions. TIP1 expression, for example, is induced at high- and low temperatures (Kondo and Inouye 1991) and during anaerobic growth (Donzeau et al. 1996). Increased TIR1 expression occurs under conditions of fermentation (Donzeau et al. 1996, Marguet and Lauquin 1986) and at low temperatures (Kowalski et al. 1995). Surprisingly, no clear phenotypes are found when any of these genes is deleted; only deletion of CWP2 results in an increased sensitivity to Calcofluor White, Congo Red, and Zymolyase (van der Vaart et al. 1995), whereas deletion of SED1 results in a somewhat increased tolerance for Calcofluor White and Congo Red (M.J. van der Vaart, personal communication). Deletion of EGT2, which encodes another cell wall protein (Caro et al. 1997) does result in a clear phenotype, namely a delayed cell separation (Kovacech et al. 1996).

We studied the transcription of these cell wall protein-encoding genes during the cell cycle. We were expecting to find a certain amount of cell cycle regulation, since incorporation of mannoproteins into the cell wall is cell cycle regulated and highest in M-phase (De Nobel et al. 1991). Surprisingly, transcription of all five genes was cell cycle regulated, and occurred in at least three different phases of the division cycle: in S/G2- (CWP1, CWP2 and TIR1), in M- (SED1) and in early G1-phase (TIP1 (and EGT2 (Kovacech et al. 1996))). By studying transcript levels, one cannot draw definite conclusions about the protein expression levels. However, recent data show that GFP-fusion proteins of Cwp1p and Cwp2p are incorporated in distinct but separate regions of the cell wall (A.F.J. Ram, unpublished results). This asymmetric distribution is consistent with cell cycle regulated expression. Possibly, asymmetric deposition of cell wall proteins provides the cells with landmarks for, for example, bud-site selection. In addition, the fact that various cell wall proteins are differentially expressed under different growth conditions, makes it highly likely that they have specific (structural) functions.

ACKNOWLEDGMENTS

We would like to thank Dr. M.J. van der Vaart who supplied us with several probes. We also thank Dr. A.F.J. Ram for sharing data prior to publication. Furthermore, we are much obliged to P. Huls, who performed the centrifugal
elutriation. This research was supported by the Dutch Ministry of Economic Affairs and the Life Science Foundation (SLW).

REFERENCES


The dynamics of cell wall biogenesis in yeast


