Regulation of Flo11p-dependent adhesion in *Saccharomyces cerevisiae*

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Summary

FLO11 is coding for a cell surface adhesin in the baker's yeast *Saccharomyces cerevisiae*. Its expression is regulated by different environmental circumstances like glucose, nitrogen or amino acid limitation. Flo11p is strictly required to allow cells to react on these nutrient signals by a dimorphic switch from single growing yeast cells to multicellular complexes with adhesive phenotype.

This work demonstrates that under repressed conditions the unusually large *FLO11* promoter of about 3 kb contains only one MNase-sensitive site located 1.2 kb upstream of the open reading frame. This site correlates with the binding region for the repressor protein Sfl1p. Investigations with genes for components involved in chromatin establishment, maintenance or remodeling identified the histone variant H2A.Z/Htz1p as yet unknown factor that is required to keep *FLO11* in a silent state. The chromatin remodeler Rsc1p and the histone acetyl transferase Gcn5p are antagonists to H2A.Z/Htz1p and are required to overcome this silent state under glucose depletion, and therefore, to switch to the adhesive growth mode or pseudohyphal development. Addition of the histidine analogue 3-aminotriazol results in amino acid starvation and restores Flo11p-dependent adhesion in Δ*rsc1* cells. These cells express only low *FLO11* mRNA levels suggesting that there might be additional mechanisms which result in sufficient amounts of adhesin molecules. These mechanisms might be regulated on a post-transcriptional level. A possible post-transcriptional level of controlling *FLO11* expression was addressed by analysing two isogenic ribosomal proteins, namely Rps26Ap and Rps26Bp. Both proteins are compounds of the small subunit of the ribosome and are involved in regulating *FLO11* expression. Only Rps26Ap is an essential factor for efficient *FLO11* mRNA translation. Investigations concerning the regulation of the two isogenes demonstrate a reciprocal effect on the translational level. Rps26Ap stimulates the translation of *RPS26B* mRNA into the protein, whereas formation of Rps26Ap is inhibited by Rps26Bp.

Zusammenfassung

In der Bäckerhefe *Saccharomyces cerevisiae* kodiert *FLO11* für ein Zell-Oberflächenprotein. Dessen Expression wird von verschiedenen Umweltbedingungen, wie Glukose-, Stickstoff- oder Aminosäuremangel reguliert. Um auf diese Nährstoffsignale reagieren zu können wird Flo11p benötigt, damit Zellen von einer einzelligen Hefeform zu einem multizellulären Komplex wechseln können. Dieser zeichnet sich durch einen adhäsiven Phänotyp aus.

Diese Arbeit zeigt, dass unter reprimierten Bedingungen der ungewöhnlich große *FLO11*-Promotor mit einer Länge von etwa 3 kb durch eine Reihe von Nukleosomen bedeckt ist, die durch eine MNase-sensible Stelle unterbrochen wird. Diese befindet sich etwa 1.2 kb stromaufwärts des offenen Leserahmens und korreliert mit der Bindestelle für das Repressor-Protein Sfl1p. Untersuchungen an Genen, die für Komponenten kodieren, die an dem Aufbau von Chromatin, dessen Erhaltung oder der Umgestaltung beteiligt sind, zeigten, dass die Histonvariante H2A.Z/Htz1p an der Erhaltung des stillgelegten Zustands von *FLO11* beteiligt ist. Die Chromatin-verändernde Komponente Rsc1p und die Histon-Acetyltransferase Gcn5p sind Gegenspieler von H2A.Z/Htz1p und deshalb notwendig, um diesen stillgelegten Zustand von *FLO11* unter Glukosemangel zu überwinden und dadurch zu der adhäsiven Wachstumsform zu wechseln. Die Zugabe von 3-Aminotriazol, einem Histidin-Analogon, führt zu Aminosäure-Mangel und stellt die Flo11p-abhängige Adhäsion in Δ*rsc1* Zellen wieder her. Diese Zellen zeigen nur sehr geringe *FLO11* mRNA Mengen, was auf zusätzliche Mechanismen für eine ausreichende Menge an Adhäsinen schliessen lässt. Diese Mechanismen scheinen auf post-transkriptioneller Ebene abzulaufen. Eine dieser post-transkriptionellen Ebenen, die die *FLO11* Expression kontrolliert, wird durch zwei fast gleiche ribosomale Iso-Proteine vermittelt, Rps26Ap und Rps26Bp. Beide Proteine sind Komponenten der kleinen Untereinheit des Ribosoms und an der Regulation der *FLO11* Expression beteiligt. Dabei ist nur Rps26Ap für eine effiziente Translation der *FLO11* mRNA nötig. Untersuchungen zur Regulation der beiden kodierenden Isogene zeigen eine gegenseitige Kontrolle auf translationeller Ebene.

Chapter 1

Introduction

Regulation of gene expression enables organisms to react to environmental stimuli. Some of the genes on the genome have to be frequently or strongly expressed, whereas others are subject to a rare or slight transcription. The regulational network of the cell is tightly complex. It includes sensors to relay environmental stimuli, and signal transduction pathways that finally result in transcriptional activators or repressors. Thereby, different pathways are networked with each other. Furthermore, the consistence of the DNA becomes important in regulating initiation of gene expression (Yuan *et al.*, 2005). Maturation of the eukaryotic RNA, including splicing, editing, stability and transport into the cytoplasm affects regulation of gene expression on a post-transcriptional level (Gray and Wickens, 1998; Zorio and Bentley, 2004). In addition, translational regulation including initiation of translation was found to control some mRNAs and as a consequence the activity of some proteins (Vilela and McCarthy, 2003). Protein stability and transport of the protein to the correct target compartment also might impact the functionality and have to be taken into account.

1. Chromatin structure as regulating factor of gene expression in eukaryotes

1.1 Chromatin and nucleosome structure of the eukaryotic DNA

Chromosomal DNA differs in formation between eukaryotes and prokaryotes. Whereas genes in a prokaryotic genome are apparently instantly accessible to ensure a rapid response to environmental change, the majority of chromosomal DNA in the

eukaryotic nucleus is inactive, packaged in chromatin. Examples of histone-based DNA packaging have also been found in the prokaryotic domain archaea, which form one such family of architectural chromosomal proteins (Reeve *et al.*, 2004; Sandman and Reeve, 2000). Eukaryotic chromatin is organized in a hierarchy of structures, starting with the nucleosome as basic repeat unit, and resulting in a highly condensed complex appearance of metaphase chromosomes (Wolffe, 1995). This compactness of the eukaryotic chromosome consisting of condensed chromatin fibres is required to fit DNA of a length of more than a meter within the limited volume of the nucleus. The nucleosome consists of an octameric protein complex, which is surrounded by about 120 to 200 bp of DNA. The DNA wraps in 1.65 left-handed turns around the protein core (Luger *et al.*, 1997), which is composed of two of each histone proteins H2A, H2B, H3 and H4 (Figure 1A). The amino-terminal histone tails extend from the nucleosomal core. These positively charged regions are targets of reversible post-transcriptional modifications, which alter either their charge or their conformation, and therefore, the interaction with DNA or other chromatin associated proteins, which recognize DNA sequences with little or no sequence specificity (Grosschedl *et al.*, 1994) (Figure 1A).

The linker DNA connects the nucleosomes resulting in a structure like beads on a chain. During metaphase these structures condense to the chromatin in which form DNA has to fit into the nucleus (Figure 1B). This highly ordered structure is effective in gene repression because transcription and all other nuclear processes require that enzymes and transcription factors gain access to the DNA template. The presence of nucleosomes restricts the access of proteins to the DNA, and therefore, suppresses gene expression (Lorch *et al.*, 1999; Workman and Roeder, 1987). Mapping of chromatin structure demonstrates that DNA is not densely packed in nucleosomes, but especially regulatory elements appear as nucleosome-free regions. In general, active genes exhibit nucleosome-free regions in their 5' domain, whereas the corresponding regions of inactive genes seem to be densely packed into nucleosomes (Elgin, 1988). Therefore, regulated genes require remodeling of the chromatin structure in their regulatory region to assist or repress the transcription by binding of certain transcription factors or other mechanisms that are involved in gene transcription (Wallrath *et al.*, 1994).

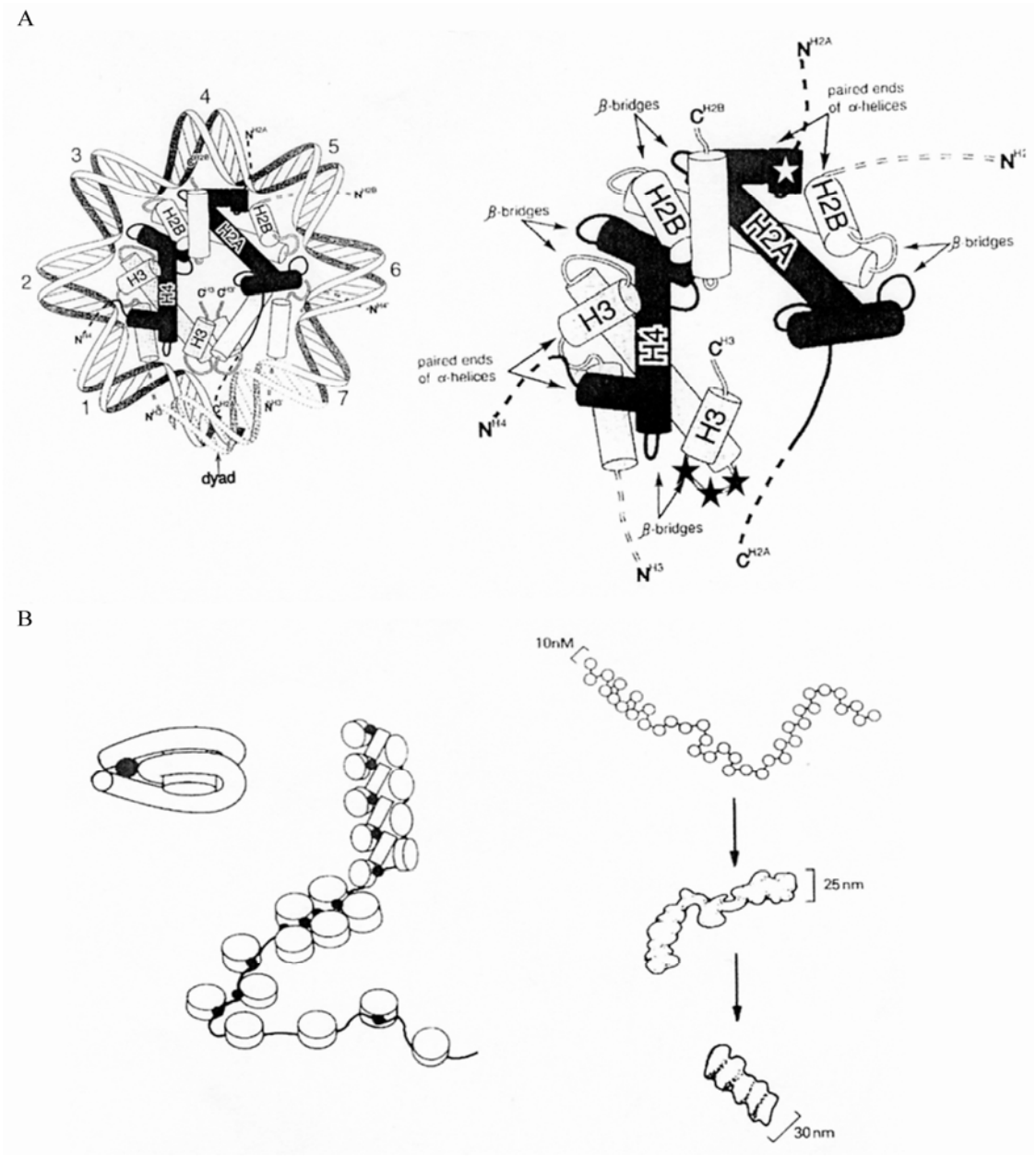

Figure 1. Nucleosome structure and levels of chromatin packing. A. Right: The structural model of a nucleosome displays the interaction of the core histones with DNA. The DNA is not shown and the view corresponds to one turn of the DNA. For clarity only one molecule of H2A, H2B, and H4, respectively, is shown. Left: The scheme shows interactions between heterodimers of H2A, H2B, and H3-H4, and DNA. The sites of primary interaction of the histone fold domains with DNA (the paired ends of helices and L-bridge motifs) are indicated. **B.** The left model presents a nucleosome with associated proteins (black ball). In the middle the 'beads-on-a-string' form of chromatin is shown. At right are folding intermediates from 'beads-on-a-string' form to the 30 nm flat fibre. Adapted from Wolffe (1995).

1.2 Chromatin structure regulates gene expression

Besides the sequence of the promoter region of the expressed gene, and certain upstream binding sites, which are recognised by specific transcription factors, the chromatin structure can function as inhibitor of gene transcription.
Generally, chromatin exists in different states, the transcriptionally silenced heterochromatin and the actively transcribed euchromatin. Transcriptional silencing through heterochromatin is a heritable form of gene inactivation that involves the assembly of large regions of DNA. This silent chromatin is present around centromeres and telomeres and among chromosome segregation and genomic stability (Huang, 2002; Wallrath, 1998). A prominent example of this kind of transcriptional silencing is the presence of intact but unexpressed copies of genes. In yeast the mating type loci *HML* and *HMR* are embedded in a heterochromatic structure (Weiss and Simpson, 1998). A classic example of transcriptional silencing in higher eukaryotes is the inactivation of one of the heterochromatic X-chromosomes in female mammals (Cohen *et al.*, 2005; Heard *et al.*, 1997). The structure of euchromatin imposes a default repressed state upon the genome. Changing in this chromatin structure regulates the expression of certain genes.

1.2.1 Chromatin modifying activities enable transcriptional regulation

Activation or repression of transcription requires binding of certain transcription factors or other mechanisms which are involved in gene transcription (Wallrath *et al.*, 1994). During gene regulation, the nucleosome structure has to be destabilized in order to facilitate the access of sequence-specific binding factors and the general transcription-machinery. Two major classes of complexes are known to regulate accessibility of the template to DNA binding factors. One class includes ATP-dependent complexes, which can move positioned nucleosomes. The other class of complexes modifies histone proteins by acetylation, phosphorylation, ubiquitination, or methylation (Berger, 2001). Both mechanisms change DNA packaging, and therefore, DNA accessibility. Gene specific transcription often depends on the two

most important chromatin remodeling systems, including the ATP-dependent SWI/SNF-like complex, which moves nucleosomes (Mohrmann and Verrijzer, 2005), and histone acetylation or deacetylation machineries, which modify histones (Khan and Krishnamurthy, 2005).

1.2.1.1 ATP-dependent remodeling complexes

ATP-dependent remodeling complexes facilitate transcription regulation by regulating the access of nucleosomal DNA to various transcription factors. The energy of ATP hydrolysis is required to enhance this accessibility. The common subunit of these complexes is the ATPase that associates with different additional proteins. Two well characterized families of remodeling complexes in yeast are the SWI2/SNF2 family and the ISWI (imitation SWI) group (Eisen *et al.*, 1995; Sif, 2004). Among others, the SWI2/SNF2 family includes the SWI/SNF and the RSC complex of yeast. Many subunits of SWI/SNF in yeast were identified as positive regulators of transcription (Winston and Carlson, 1992). The RSC complex contains many proteins that are homologues of SWI/SNF subunits, however, the RSC complex is far more abundant than SWI/SNF in yeast cells (Figure 2). Isw1p and Isw2p are members of two distinct multisubunit remodeling complexes and assigned to the ISWI family (Dirscherl and Krebs, 2004; Tsukiyama *et al.*, 1999) (Figure 2).
The mechanism of remodeling complexes to expose nucleosomal DNA is sliding of the DNA with respect to the histone octamer (Meersseman *et al.*, 1992; Sif, 2004). This results in a translationally repositioned octamer, and therefore, in nucleosome-free DNA, which originally interacted with histones. Sliding mechanisms alter the location of exposed DNA, and will not increase the amount of exposed DNA.

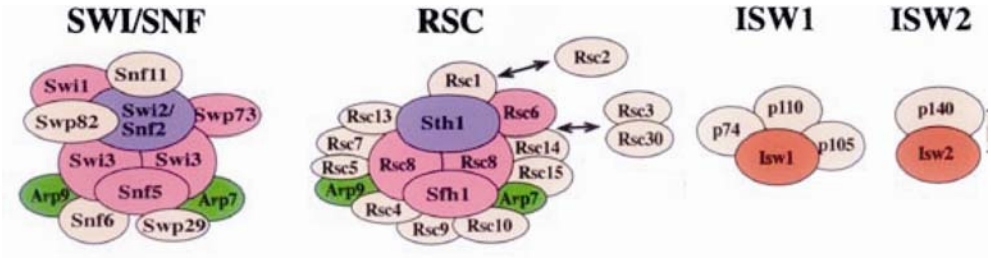

Figure 2. ATP-dependent chromatin remodeling complexes. Swi2/Snf2-related ATPases are indicated in purple, ISWI-related ATPases are depicted in red. Subunits conserved between Swi/Snf complexes are shown in pink, while subunits specific to each complex are indicated in peach. Actin-related proteins (Arp) are shown in green. Adapted from Sif (2004).

1.2.1.2 Covalent modifications of histone tails correlate with gene transcription

The positively charged regions of the amino-terminal histone tails are targets of reversible post-transcriptional modifications that include acetylation, methylation, and ubiquitination of specific lysine amino acids, or phosphorylation of serines. Beyond these different modifications of the histone core, acetylation is the best investigated alteration with respect to gene transcription. The level of acetylation is maintained by the opposite actions of histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes (Reid *et al.*, 2000; Vogelauer *et al.*, 2000). Histone acetyl transferases (HATs) catalyze the transfer of an acetyl group from acetyl-CoA to the acceptor amino group of lysine chains within the amino-terminal tails of the core histones H2A, H2B, H3, and H4. This acetylation neutralizes parts of the positive charged tail region, resulting in a weaker histone-DNA (Hong *et al.*, 1993; Steger and Workman, 1996) or nucleosome-nucleosome interaction (Fletcher and Hansen, 1996; Luger and Richmond, 1998). This destabilizes chromatin folding, and thereby, other nuclear factors gain access to DNA, resulting in enhanced gene transcription (Bauer *et al.*, 1994). Increased acetylation in proximal promoter regions correlates with recruitment of HAT complexes, and therefore, with increased gene expression (Kuo *et al.*, 2000). Similarly, hypoacetylation represses gene expression,

and correlates with recruitment of HDAC complexes (Bulger, 2005; Khochbin *et al.*, 2001). A well characterized component of complexes with HAT activity is Gcn5p, which is conserved from yeast to humans (Grant *et al.*, 1997). Gcn5p is the catalytic subunit of at least two distinct large multisubunit protein complexes, ADA (for Adaptor) and SAGA (Spt/Ada/Gcn5 acetyltransferase). They are targeted to gene promoters by transcriptional activators, and facilitate the activation of inducible genes (Pollard and Peterson, 1997). Additionally, Gcn5p is involved in the maintenance of a basal level of genome-wide histone acetylation in a not yet understood manner (Krebs *et al.*, 1999; Kuo *et al.*, 2000; Vogelauer *et al.*, 2000).

1.2.1.3 Variant histone genes

In addition to histone modifications and nucleosome remodeling processes, there is a further form of chromatin regulation that involves the replacement of canonical histones with histone variants. A famous histone variant is H2A.Z, which replaces H2A in about 10 % of the nucleosomes. H2A.Z is highly conserved between yeast and human (Jackson *et al.*, 1996; Santisteban *et al.*, 2000).
H2A.Z differs from H2A in the C-terminal region by an acidic patch extending to H2B (Suto *et al.*, 2000). The crystal structure of a H2A.Z containing nucleosome reveals an altered surface of the nucleosome: it includes a metal ion, that creates a potential surface mediating novel interactions between H2A.Z and other nuclear proteins by changing the higher order structure of chromatin (Suto *et al.*, 2000). In *S. cerevisiae* H2A.Z prevents the spreading of heterochromatin into adjacent euchromatic regions like telomeres (Meneghini *et al.*, 2003).

1.3 Repression and activation of non-silenced chromatin

Gene expression is regulated on different levels. The local arrangement of the nucleosomal structure regulates the interaction of DNA-binding proteins to the DNA, including the transcription machinery (Grewal and Moazed, 2003). Chromatin

modifying activities like SWI/SNF complex or HATs (Histone-acetyltransferases) are necessary to change the epigenetically silenced chromatin into a DNA structure, which can be repressed or activated through sequence-specific transcription factors (Khan and Krishnamurthy, 2005; Sif, 2004). These DNA-binding factors, including both activators and repressors assist or inhibit the transcription of certain genes (Figure 3). Furthermore, gene expression arranged through binding factors depends on transport and stability of the factors themselves.

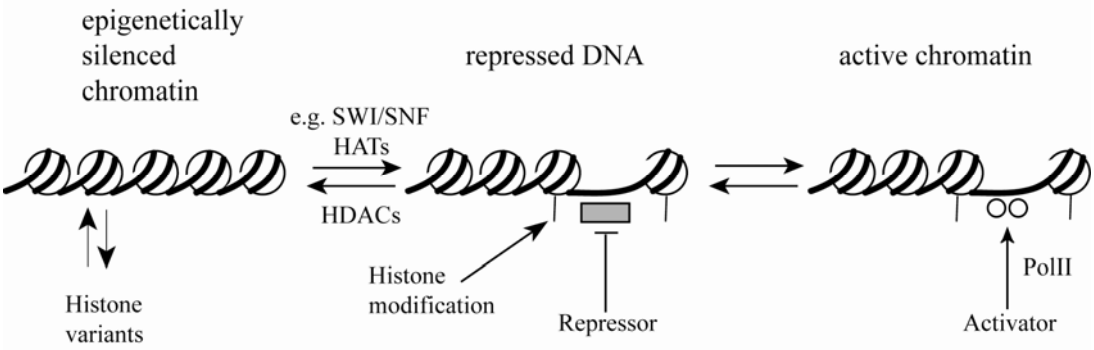

Figure 3. Different nucleosomal structures and the corresponding gene expression. The epigenetically silenced chromatin structure can be modified by altering the structure through chromatin remodeling complexes like SWI/SNF or modifying complexes with histone-acetyltransferase activities (HATs). Histone-deacetyltransferase activities (HDACs) remove acetyl-groups from the histones. Thereby, DNA presents certain sequences for binding factors which repress or activate gene expression.

1.3.1 Transcriptional initiation and its regulation in yeast

Transcription of DNA into RNA requires an orchestration of basal and regulatory transcription factors (Chadick and Asturias, 2005; Reinberg *et al.*, 1998; Roeder, 1998). The rate of transcription initiation is a very important step which defines the level of gene expression (Struhl, 1995; Weber *et al.*, 1977). One important mechanism of controlling transcription initiation depends on the promoter region of the expressed gene, including the TATA binding box (Cormack and Struhl, 1992;

Schultz *et al.*, 1992), and other upstream binding sites that are recognised by specific transcription factors.

The TATA binding box is necessary for an efficient transcription initiation (Stewart and Stargell, 2001). In *S. cerevisiae* a major consensus sequence of the TATA binding motif (TATA(A/T)A) was found, whereas variations within this binding site can regulate gene expression by differences in efficiency of binding of the RNA pol II complex (Stewart and Stargell, 2001). The TATA binding protein (TBP) is associated with several TAFs (TBP-associated factors) resulting in the TFIID complex (Struhl, 1997; Yatherajam *et al.*, 2003). This complex forms the starting point together with the RNA pol holoenzyme complex and directs the RNA polymerase to the transcriptional start site by interacting with different RNA pol II subunits (Stargell *et al.*, 2000).

A further efficient control of transcription initiation is mediated by certain transcription factors (Struhl, 1995). *Trans*-acting proteins direct the RNA pol II complex to a specific transcriptional start point or they prevent the binding, and therefore, the transcription initiation (Ullmann *et al.*, 1967). Specific *cis*-elements within the upstream region of the controlled genes bind certain transcription factors. Depending on the agency of the transcription factor, these elements are upstream activation sites (UAS) or upstream repression sites (URS). Certain mediator proteins direct the complex association of all *cis*- and *trans*-acting factors involved in transcription initiation (Boube *et al.*, 2002; Lewis and Reinberg, 2003).

As mentioned before, the chromatin structure also plays an important role in regulation of gene expression. A highly condensed state of chromatin structure prevents access of transcription factors and the RNA pol II complex to the DNA, and therefore, the initiation of gene transcription, whereas a heterochromatic structure of DNA is completely silenced.

2. Ribosomes - Structure and Translation

2.1 Eukaryotic ribosomes

RNA-based translation in eukaryotes occurs at 80S ribosomes. Translation of one mRNA proceeds simultaneously at multiple ribosomes, which forms a polysome structure (Slayter *et al.*, 1963). The eukaryotic ribosomes are composed of a small 40S subunit and a large 60S subunit (Green and Noller, 1997; Spahn *et al.*, 2001) (Figure 4). The small subunit (40S) comprises the 18S rRNA and 33 proteins, whereas the large subunit (60S) compounds approximately 50 proteins and 28S, 5.8S, and 5S rRNAs (Spahn *et al.*, 2001). The yeast ribosome differs from those of other eukaryotic ribosomes, as e.g. mammals. The large subunit only includes 46 proteins and the small subunit contains 32 proteins. Additionally, the yeast ribosome contains a 25S rRNA within the 60S subunit instead of the 28S rRNA in other eukaryotic ribosomes (Verschoor *et al.*, 1998) (Figure 4).

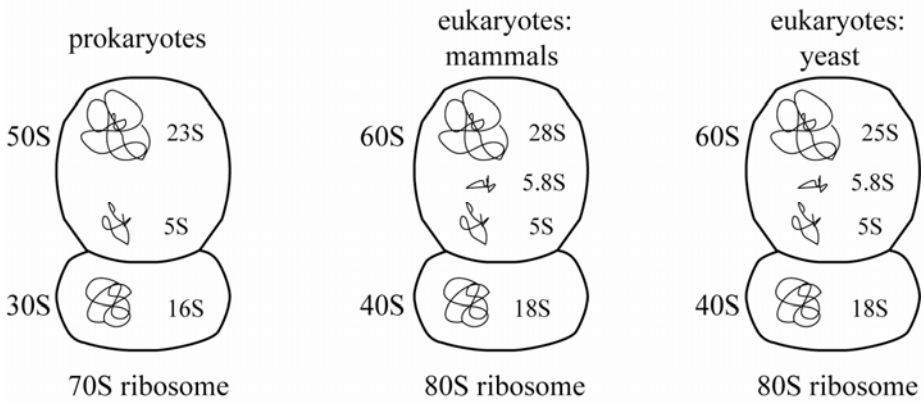

Figure 4. Ribosomes of different organisms. Prokaryotic ribosomes sediment with 70S and are composed of a 50S and a 30S subunit (Gabashvili *et al.*, 2000; Wimberly *et al.*, 2000), whereas eukaryotic ribosomes sediment with 80S and contain the subunits 60S and 40S (Green and Noller, 1997; Spahn *et al.*, 2001). In contrast to prokaryotes, eukaryotic ribosomes contain an additional rRNA that sediments at 5.8S (Spahn *et al.*, 2001). Yeast ribosomes include a 25S rRNA in the large subunit instead of a 28S rRNA in other eukaryotes (Verschoor *et al.*, 1998).

2.1.1 Ribosomal proteins in yeast

The yeast genome carries 137 genes encoding ribosomal proteins. 32 of them are different proteins of the small subunit of the ribosome, 46 are large-subunit proteins (Planta and Mager, 1998). The ribosomal protein genes (*rp*-genes) are scattered all over the genome of *S. cerevisiae*. Often *rp*-genes are duplicated and contain an intron (Raue *et al.*, 1991). The proteins encoded by duplicated *rp*-genes usually differ by no more than one or two amino acids. As example the amino acid sequence of the two ribosomal proteins Rps26Ap and Rps26Bp is given in Figure 5. The proteins differ in two of the 119 codons at position 106 and 113, respectively. The non-coding regions of most duplicated ribosomal genes, including the introns, have diverged almost completely.

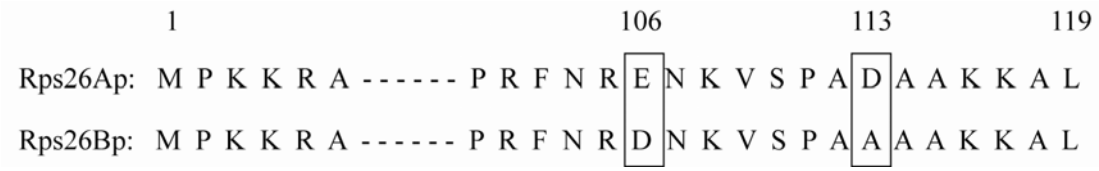

Figure 5. Differences between the Rps26A and the Rps26B protein of the small subunit of the ribosome in *Saccharomyces cerevisiae*. Positions relative to the N-terminal ATG start codon are indicated by the numbers above the amino acids. The deduced Glu^{106} and Asp^{113} residues of Rps26Ap are substituted for Asp^{106} and Ala^{113} in Rps26Bp.

Rp-gene promoters are highly efficient, so that the corresponding transcripts account for about 20 % of the total mRNA in the cell (Velculescu *et al.*, 1997). Transcription of most of the *rp*-genes in yeast is mediated by the DNA-binding protein Rap1p (Mager and Planta, 1990), and the forkhead-like transcription factor Fhl1p (Lee *et al.*, 2002). Additionally, some of the *rp*-gene promoters contain one single binding site for Abf1p (ARS- (autonomously replicating sequence) binding factor), a global regulator (Mager and Planta, 1990). Abf1p plays roles in ARS activity during DNA replication, transcriptional activation and transcriptional silencing of *HM* mating-loci

(Rhode *et al.*, 1992). Furthermore, a characteristic T-rich region serves as auxiliary element in reaching the high transcriptional rate of *rp*-genes (Buchman and Kornberg, 1990; Goncalves *et al.*, 1995). This T-rich region plays primarily a structural role. They may keep the promoter nucleosome-free, and therefore, facilitate the access of the transcriptional machinery to the promoter (Struhl *et al.*, 1985).

2.2 Translational regulation in eukaryotes

Translational regulation in eukaryotes takes place in the cytoplasm, and therefore, the mRNAs have to be exported from the nucleus. The efficiency of translation depends on the control of translation initiation, and on stability and degradation of the respective mRNA (Day and Tuite, 1998). The stability of mRNAs is controlled by different mechanisms. These are specific intrinsic nucleotide sequences and RNA-binding proteins, which bind either many mRNAs or mRNA specific. The 5' cap and the 3' poly(A) structures of eukaryotic mRNAs have important roles in promoting mRNA stability in the nucleus, its transport to the cytoplasm, and its translation and stability in the cytoplasm (Searfoss and Wickner, 2000). The 3' poly(A) tail protects degradation by 5' decapping and subsequent 5' $\rightarrow$ 3' degradation by Xrn1p/Ski1p (Decker and Parker, 1994). The majority of nonsense transcripts are recognized and efficiently degraded via the NMD-pathway (nonsense-mediated mRNA decay). This protects the organism from negative or gain-of-function effects of truncated proteins that could result if nonsense transcripts were stable. In general, the decay of mRNA involves deadenylation-dependent 5'-decapping and subsequent 5' $\rightarrow$ 3' decay by the Xrn1p exonuclease (Caponigro and Parker, 1996; Decker and Parker, 1994; Frischmeyer and Dietz, 1999). The half-life of mRNAs is also dependent on developmental or environmental stimuli, such as nutrient levels, hormones, or temperature shifts.

The translation initiation is regulated by modifications of the eukaryotic initiation factors (eIF) (Day and Tuite, 1998; Valente and Kinzy, 2003), as well as the

structural features of the 5' UTR, i.e. the poly(A) tail, which is essential for translation (Proweller and Butler, 1994). Translation in eukaryotes requires a functional 80S initiation complex, including the small 40S ribosomal subunit bound to mRNA, the initiation tRNA (Met-tRNAi), as well as the 60S ribosomal subunit. This 80S ribosome formation is mediated by different eukaryotic initiation factors (eIFs). First, a 43S pre-initiation complex has to be built by binding of an eIF2/GTP/Met-tRNAi ternary complex and other eIFs to the 40S subunit. This 43S pre-initiation complex binds to the mRNA and moves along the 5' non-translated region from its initial binding site to the initiation start codon (AUG). The anti-codon of the initiator tRNA base paired to the AUG of the mRNA, resulting in the 48S initiation complex. After dissociation of eIFs and binding of the 60S subunit, a functional 80S ribosome is performed (for details see 2.1.), which is ready for translational elongation.

The initiation of translation is either globally regulated or mRNA specific. The global regulation results from modifications of the eIFs, mostly of phosphorylation. The mRNA specific translational regulation depends often on the structural feature of the transcript. Certain structures can inhibit initiation by impeding 40S subunit binding or scanning or by acting as receptor for regulatory RNA binding proteins. Furthermore, the presence of upstream AUGs highly regulate the translational initiation at the originally start codon. These out-of-frame AUGs prevent initiation at the downstream AUG codon, and therefore, inhibit translation. A closer position to the authentic AUG codon compounds the inhibition of translation, independent of a termination codon in frame of the uAUG (Yun *et al.*, 1996). An exception is the *GCN4* mRNA with four uORFs (Hinnebusch, 1984, 1994), where initiation is coupled to the efficiency of termination (Grant and Hinnebusch, 1994).

3. Cellular differentiation in dependence on nutritional signals in yeast

Dimorphism, the switch from unicellular to multicellular growth type is widely spread in pathogenic organism. It is an important virulence factor, determining

invasion and colonization the host by pathogenic fungi like *Candida albicans* and *Ustilago maydis* (Lengeler *et al.*, 2000; Sanchez-Martinez and Perez-Martin, 2001). Bacteria also achieve pathogenicity by adherence, for instance the human pathogens *Pseudomonas aeruginosa* or *Salmonella typhimurium*, leading to affection of the gastrointestinal tract.

Beyond many pathogenic fungi, the budding yeast *S. cerevisiae* can also interconvert between unicellular and multicellular growth type. Its growth modus is under the control of the available nutrients. Therefore, *S. cerevisiae* is a good model organism that may help to get new insights in pathogenicity of fungi.

3.1 Life cycle of *S. cerevisiae*

Baker's yeast exists in two haploid mating types, termed a and α. These two cell types can conjugate together to form diploid a/α cells (Herskowitz, 1989) (Figure 6). When cells are growing on media containing a fermentable carbon source and sufficient nitrogen, haploid cells show a unicellular ellipsoid morphology, called yeast form (YF). The same morphology can be observed when diploid cells are cultivated in rich nutritional medium. Depletion of one of these two nutrients leads to growth arrest (carbon starvation) or induction of filamentous growth (nitrogen starvation) in diploid cells. This growth modus form chains of elongated cells called pseudohyphae (PH) that allow diploid yeast to forage for nutrients in a starving colony (Gimeno *et al.*, 1992). Pseudohyphal cells exhibit invasive growth directly into the substrate. In haploid cells that are starved for a nutritional signal, a relative phenomenon can be observed, the haploid invasive growth. This phenotype is characterized by surface adhesion with developing small microfilaments (Cullen and Sprague, 2000). The cells are not completely separated from each other, and therefore, form multicellular complexes. Additionally, when diploid cells starved for both, glucose and ammonium as nutritional signals, they will undergo meiosis (Figure 6).

Dimorphism, the switch from the yeast form to filamentous growth type is subject to several distinct cellular processes. In diploid as well as in haploid cells, the budding modus of cells changes from a bipolar or axial to a unipolar distal pattern (Taheri *et al.*, 2000) (Figure 6). Furthermore, cells remain attached to each other after budding resulting in linear filamentous cell chains (Gimeno *et al.*, 1992). Diploid pseudohyphal cells exhibit invasive growth with direct invasion into the substrate, whereas diploid cells growing in the yeast form do not. In contrast to diploids, haploid cells exhibit only moderately elongated cells, when they switch from yeast form to filamentous growth.

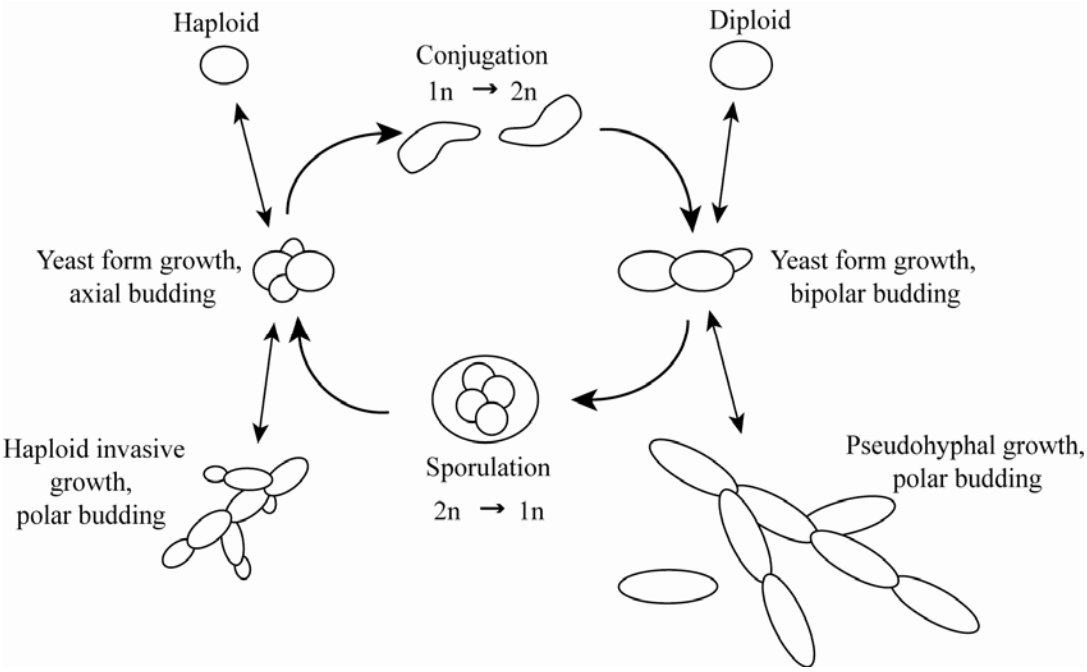

Figure 6. Life cycle of *Saccharomyces cerevisiae*. Haploids (1n) of opposite mating type can conjugate to form diploids (2n), and these diploids can sporulate to form haploids. Both haploid and diploid *S. cerevisiae* cells can grow vegetatively in the yeast form or switch to an invasive growth mode. Invasively growing diploids develop multicellular filaments called pseudohyphae, whereas haploids only produce microfilaments. Adapted from (Mösch, 2000).

3.2 The *FLO*-family of adhesins of *Saccharomyces cerevisiae*

Adhesins, a family of cell wall glycoproteins, are required for interaction of fungal cells with each other (Guo *et al.*, 2000; Lo and Dranginis, 1998; Teunissen and Steensma, 1995), surfaces like agar or plastic (Gaur and Klotz, 1997; Lo and Dranginis, 1998; Reynolds and Fink, 2001), and mammalian tissues (Cormack *et al.*, 1999; Hazen *et al.*, 1991; Li and Palecek, 2003). Also the formation of biofilms depends on fungal adhesins (Baillie and Douglas, 1999; Green *et al.*, 2004; Reynolds and Fink, 2001). The structure of all adhesins is modularly configured and consists of three domains, A, B, and C (Figure 7). Domain A at the N-terminus is thought to provide much of the affinity of the adhesins for surfaces (Kobayashi *et al.*, 1998). This segment is followed by domain B, which is variable in length and extremely rich in serine and threonine residues. The carboxy-terminal domain C is conserved among *FLO1*, *FLO5*, and *FLO9* and contains a site for the covalent attachment of a glycosyl-phosphatidylinositol anchor (Verstrepen *et al.*, 2004).

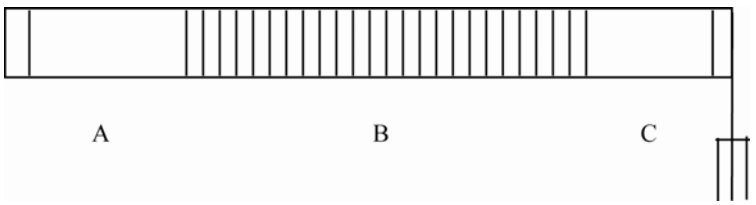

Figure 7. Domain structure of yeast adhesins. Adhesins comprise three domains, A, B, and C, which are preceded by an amino-terminal signal sequence. The amino-terminal domain A is thought to confer adhesion. The central domain B contains a serine/threonine-rich region that is encoded by many repeated nucleotide sequences. The carboxy-terminal domain C contains a site for the covalent attachment of a glycosyl phosphatidylinositol anchor. Adapted from Verstrepen *et al.* (2004).

In *S. cerevisiae*, *FLO* genes confer adhesion only to agar, plastic and other yeast cells (Guo *et al.*, 2000), and not to mammalian tissues like *Candida albicans* or *Candida glabrata* do (Cormack *et al.*, 1999; Hazen *et al.*, 1991; Li and Palecek, 2003). Adherence to mammalian tissues of these pathogens depends on proteins encoding

by *ALS* gene family (*Candida albicans*) or the *EPA* genes (*Candida glabrata*), respectively (Cormack *et al.*, 1999; De Las Penas *et al.*, 2003; Hoyer, 2001). Five members of the *FLO* gene family (*FLO1*, *FLO5*, *FLO9*, *FLO10*, and *FLO11*) are known in *S. cerevisiae*, whereas in the Σ1278b genetic background the only expressed member is *FLO11*. In contrast to *FLO11*, the other *FLO* genes are transcriptionally silent (Guo *et al.*, 2000). They are located adjacent to their respective telomeres (~ 10 to 40 kb) that might be important for their expression and genetic interaction (Halme *et al.*, 2004; Teunissen and Steensma, 1995).

3.3 Adhesion and invasive growth in yeast depend on *FLO11*, an adhesin encoding gene

Under certain nutrient starvation conditions diploid cells, as well as haploid yeast cells exhibit cell-cell-adhesion, and adhesion to solid substrates. Diploid cells show enhanced adhesiveness due to the pseudohyphal filaments, which grow down into the medium. The pseudohyphal growth form, as well as invasive growth may enable the non-motile yeast to forage for nutrients under adverse conditions. In the laboratory, yeast adhesion and invasive growth can be assayed by washing the plate with water. In the case of diploids, pseudohyphal cells cannot be washed away even when rubbed on the plate. In contrast, non-adhesive yeast form cells can easily be washed away.

Beside flocculation and biofilm formation, cell-substrate adhesion, invasive growth and pseudohyphal development requires the expression of the glycosyl-phosphatidylinositol (GPI)-liked cell surface flocculin *FLO11* (Guo *et al.*, 2000; Lambrechts *et al.*, 1996; Lo and Dranginis, 1996, 1998). Mutant strains lacking *FLO11* are unable to develop filaments in both haploids and diploids, and therefore, exhibit a non-adhesive phenotype. *FLO11* is hardly expressed when yeast cells are growing in the single cell yeast form, and its induction perform cell-cell and cell-surface interactions (Caro *et al.*, 1997; Guo *et al.*, 2000). Expression of *FLO11* is under the control of an unusually large promoter of at least 2.8 kb (Rupp *et al.*, 1999), whereas the calculated average length of a yeast promoter is 309 base pairs

(Dujon, 1996). *FLO11* expression is highly regulated by a complex network of signaling pathways and transcription factors. This includes the nutrient-responsive mitogen-activated protein kinase (MAPK) cascade, and the cyclic AMP-dependent protein kinase A (PKA) pathway (Gagiano *et al.*, 2002; Gancedo, 2001; Lengeler *et al.*, 2000) that mediate nutritional signals to transcription factors such as Tec1p, Ste12p and Flo8p (Liu *et al.*, 1993; Madhani and Fink, 1997; Roberts and Fink, 1994). Further signaling elements or regulators, which are associated with pseudohyphal differentiation and invasive growth are cell cycle regulators, in particular the G_1 cyclin (Ahn *et al.*, 2001; Loeb *et al.*, 1999; Oehlen and Cross, 1998), the amino acid specific response pathway (Braus *et al.*, 2003), and the meiosis-specific regulator Rme1p (van Dyk *et al.*, 2003).

3.3.1 Signal transduction pathways and transcriptional control during filamentous growth

The regulatory network that mediates filamentous growth is very complex and involves several parallel pathways, including the cAMP-dependent protein kinase A (PKA) pathway and a highly conserved mitogen-activated protein kinase (MAPK) cascade (Gancedo, 2001; Gustin *et al.*, 1998; Kronstad *et al.*, 1998; Lengeler *et al.*, 2000; Mösch, 2000; Palecek *et al.*, 2002; Pan *et al.*, 2000; Posas *et al.*, 1998) (Figure 8).

Both pathways share the small GTP-binding protein Ras2p. An activated Ras2p stimulates the adenylyl cyclase Cyr1p, and therefore, elevating the intracellular cAMP level. cAMP in turn activates the protein kinase A, a complex, which consists of one of three catalytic subunits, namely Tpk1-3p, and a single cAMP-binding regulatory subunit, Bcy1p (Broach, 1991). Although, all three Tpk proteins are redundant for viability, only Tpk2p is required for activating filamentous growth and pseudohyphal development (Robertson and Fink, 1998; Robertson *et al.*, 2000). Targets of PKA are the transcription factors Sfl1p and Flo8p, which compete on regulating *FLO11* transcription. Flo8p activates its expression (Pan and Heitman,

1999; Rupp *et al.*, 1999), whereas Sfl1p acts as repressor on *FLO11* expression (Robertson and Fink, 1998) (Figure 8).

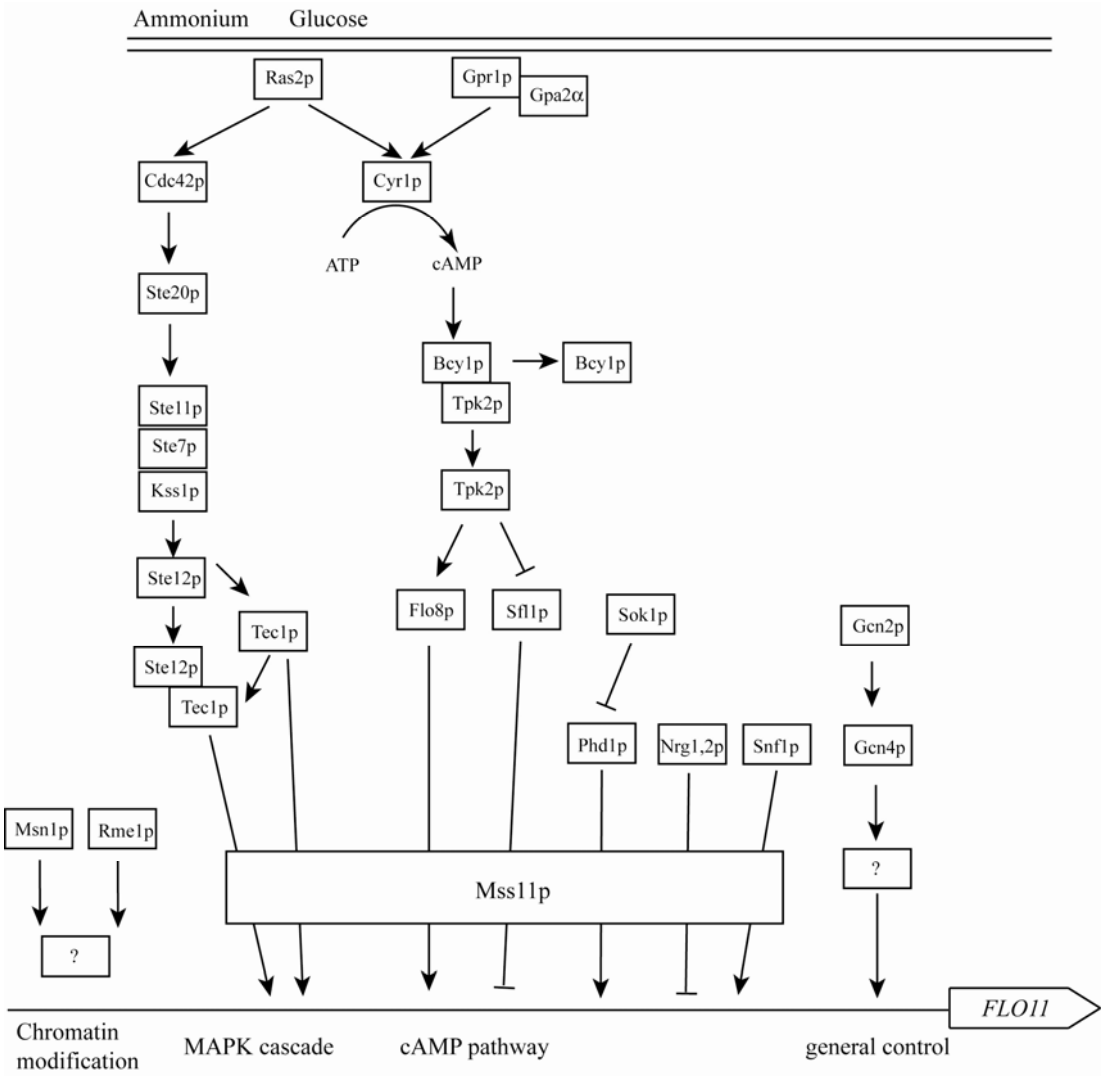

Figure 8. Model of signaling pathways regulating filamentous growth and pseudohyphal development in *Saccharomyces cerevisiae*. See text for details.

Ras2p also affects filamentous growth via another GTP-binding protein Cdc42p, which plays an essential role in regulating proliferation and and differentiation in all eukaryotes (Johnson, 1999; Mösch *et al.*, 1996), and a MAPK pathway, including the protein kinases Ste20p (MAPKKKK), Ste11p (MAPKKK), Ste7p (MAPKK), Kss1p (MAPK), and the transcription factor Ste12p (Liu *et al.*, 1993; Madhani and Fink,

1997). In an unphosphorylated state, when no signal permits filamentous growth, Kss1p binds the transcription factor Ste12p, and thereby, prevents it from activating expression of *FLO11* or other target genes (Bardwell *et al.*, 1998). Activation of Kss1p by the upstream kinase Ste7p relieves its inhibition of Ste12p that in turn activates target gene expression together with Tec1p as heterodimer (Madhani and Fink, 1997) (Figure 8). Recent studies demonstrate the requirement of a transcriptional activator, namely Mss11p, for activation of *FLO11* expression by the above-mentioned factors at the end of the signal transduction pathways (van Dyk *et al.*, 2005).

A further regulatory pathway is controlled by the 'general amino acid control' system with Gcn4p as the central element (Hinnebusch, 1997; Hinnebusch and Natarajan, 2002). Amino acid starvation leads to uncharged tRNA molecules, which bind to the sensor kinase Gcn2p (Wek *et al.*, 1995), finally resulting in phosphorylation of the translation initiation factor eIF2 and derepression of *GCN4* expression. Gcn4p acts as transcription factor of more than 500 target genes (Kleinschmidt *et al.*, 2005; Natarajan *et al.*, 2001). Amongst others, Gcn4p mediates the imbalance of amino acids to increased *FLO11* expression, and therefore, induces adhesive growth (Braus *et al.*, 2003) (Figure 8).

In addition, the highly conserved protein kinase Snf1p activates *FLO11* expression as antagonizing protein to the two repressors Nrg1p and Nrg2p (Kuchin *et al.*, 2002), as well as two putative chromatin remodeling factors Msn1p and Rme1p (Gagiano *et al.*, 1999; van Dyk *et al.*, 2003). Also involved in *FLO11* regulation is the activator encoding gene *PHD1* (Gimeno and Fink, 1994) and the associated repressor Sok2p (Ward *et al.*, 1995).

4. Aim of this work

The adhesin encoding gene *FLO11* in *S. cerevisiae* is a highly regulated gene that comprises one of the largest promoters in the yeast genome of at least 3 kb. In this work, the nucleosome structure of this gene should be mapped by *Micrococcus*

nuclease protecting experiments under conditions where *FLO11* transcription is highly repressed. Furthermore, the influence of different activities or components involved in chromatin establishment, maintenance or remodeling should be investigated with respect to the regulation of *FLO11* transcription and the resulting adhesive phenotype under certain starvation conditions. It should be analysed which components are important to maintain the silenced state of *FLO11*, as well as which factors are required to activate *FLO11* expression. Therefore, several genes coding for these activities were deleted and analysed concerning *FLO11* mRNA levels and dimorphic switch from single cell growing yeast to adhesive multicellular complexes (e.g. haploid and diploid adhesive growth or pseudohyphal development) under different environmental circumstances like glucose or nitrogen limitation, and amino acid starvation. *FLO11* expression comprises both, gene transcription and translation. Hence, the two isogenic ribosomal proteins Rps26Ap and Rps26Bp should be investigated, which are involved in Flo11p dependent adhesive growth and its regulation. In this work, the expression of *FLO11* should be determined in dependence on absence or presence of one of the two isogenes. In addition, the mutual regulation in expression of the two isogenes should be analysed.

5. References

Ahn, S.H., Tobe, B.T., Fitz Gerald, J.N., Anderson, S.L., Acurio, A., and Kron, S.J. (2001) Enhanced cell polarity in mutants of the budding yeast cyclin-dependent kinase Cdc28p. *Mol Biol Cell* **12**: 3589-3600.

Baillie, G.S., and Douglas, L.J. (1999) Role of dimorphism in the development of *Candida albicans* biofilms. *J Med Microbiol* **48**: 671-679.

Bardwell, L., Cook, J.G., Voora, D., Baggott, D.M., Martinez, A.R., and Thorner, J. (1998) Repression of yeast Ste12 transcription factor by direct binding of unphosphorylated Kss1 MAPK and its regulation by the Ste7 MEK. *Genes Dev* **12**: 2887-2898.

Bauer, W.R., Hayes, J.J., White, J.H., and Wolffe, A.P. (1994) Nucleosome structural changes due to acetylation. *J Mol Biol* **236**: 685-690.

Berger, S.L. (2001) An embarrassment of niches: the many covalent modifications of histones in transcriptional regulation. *Oncogene* **20**: 3007-3013.

Boube, M., Joulia, L., Cribbs, D.L., and Bourbon, H.M. (2002) Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. *Cell* **110**: 143-151.

Braus, G.H., Grundmann, O., Brückner, S., and Mösch, H.U. (2003) Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* **14**: 4272-4284.

Broach, J.R. (1991) Ras-regulated signaling processes in *Saccharomyces cerevisiae*. *Curr Opin Genet Dev* **1**: 370-377.

Buchman, A.R., and Kornberg, R.D. (1990) A yeast ARS-binding protein activates transcription synergistically in combination with other weak activating factors. *Mol Cell Biol* **10**: 887-897.

Bulger, M. (2005) Hyperacetylated chromatin domains: lessons from heterochromatin. *J Biol Chem* **280**: 21689-21692.

Caponigro, G., and Parker, R. (1996) Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. *Microbiol Rev* **60**: 233-249.

Caro, L.H., Tettelin, H., Vossen, J.H., Ram, A.F., van den Ende, H., and Klis, F.M. (1997) In silicio identification of glycosyl-phosphatidylinositol-anchored plasma-membrane and cell wall proteins of *Saccharomyces cerevisiae*. *Yeast* **13**: 1477-1489.

Chadick, J.Z., and Asturias, F.J. (2005) Structure of eukaryotic Mediator complexes. *Trends Biochem Sci* **30**: 264-271.

Cohen, H.R., Royce-Tolland, M.E., Worringer, K.A., and Panning, B. (2005) Chromatin modifications on the inactive X chromosome. *Prog Mol Subcell Biol* **38**: 91-122.

Cormack, B.P., and Struhl, K. (1992) The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. *Cell* **69**: 685-696.

Cormack, B.P., Ghori, N., and Falkow, S. (1999) An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* **285**: 578-582.

Cullen, P.J., and Sprague, G.F., Jr. (2000) Glucose depletion causes haploid invasive growth in yeast. *Proc Natl Acad Sci U S A* **97**: 13619-13624.

Day, D.A., and Tuite, M.F. (1998) Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *J Endocrinol* **157**: 361-371.

De Las Penas, A., Pan, S.J., Castano, I., Alder, J., Cregg, R., and Cormack, B.P. (2003) Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. *Genes Dev* **17**: 2245-2258.

Decker, C.J., and Parker, R. (1994) Mechanisms of mRNA degradation in eukaryotes. *Trends Biochem Sci* **19**: 336-340.

Dirscherl, S.S., and Krebs, J.E. (2004) Functional diversity of ISWI complexes. *Biochem Cell Biol* **82**: 482-489.

Dujon, B. (1996) The yeast genome project: what did we learn? *Trends Genet* **12**: 263-270.

Eisen, J.A., Sweder, K.S., and Hanawalt, P.C. (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res* **23**: 2715-2723.

Elgin, S.C. (1988) The formation and function of DNase I hypersensitive sites in the process of gene activation. *J Biol Chem* **263**: 19259-19262.

Fletcher, T.M., and Hansen, J.C. (1996) The nucleosomal array: structure/function relationships. *Crit Rev Eukaryot Gene Expr* **6**: 149-188.

Frischmeyer, P.A., and Dietz, H.C. (1999) Nonsense-mediated mRNA decay in health and disease. *Hum Mol Genet* **8**: 1893-1900.

Gabashvili, I.S., Agrawal, R.K., Spahn, C.M., Grassucci, R.A., Svergun, D.I., Frank, J., and Penczek, P. (2000) Solution structure of the *E. coli* 70S ribosome at 11.5 A resolution. *Cell* **100**: 537-549.

Gagiano, M., van Dyk, D., Bauer, F.F., Lambrechts, M.G., and Pretorius, I.S. (1999) Msn1p/Mss10p, Mss11p and Muc1p/Flo11p are part of a signal transduction pathway downstream of Mep2p regulating invasive growth and pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Mol Microbiol* **31**: 103-116.

Gagiano, M., Bauer, F.F., and Pretorius, I.S. (2002) The sensing of nutritional status and the relationship to filamentous growth in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **2**: 433-470.

Gancedo, J.M. (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **25**: 107-123.

Gaur, N.K., and Klotz, S.A. (1997) Expression, cloning, and characterization of a *Candida albicans* gene, *ALA1*, that confers adherence properties upon *Saccharomyces cerevisiae* for extracellular matrix proteins. *Infect Immun* **65**: 5289-5294.

Gimeno, C.J., Ljungdahl, P.O., Styles, C.A., and Fink, G.R. (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68**: 1077-1090.

Gimeno, C.J., and Fink, G.R. (1994) Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol Cell Biol* **14**: 2100-2112.

Goncalves, P.M., Griffioen, G., Minnee, R., Bosma, M., Kraakman, L.S., Mager, W.H., and Planta, R.J. (1995) Transcription activation of yeast ribosomal protein genes requires additional elements apart from binding sites for Abf1p or Rap1p. *Nucleic Acids Res* **23**: 1475-1480.

Grant, C.M., and Hinnebusch, A.G. (1994) Effect of sequence context at stop codons on efficiency of reinitiation in *GCN4* translational control. *Mol Cell Biol* **14**: 606-618.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev* **11**: 1640-1650.

Gray, N.K., and Wickens, M. (1998) Control of translation initiation in animals. *Annu Rev Cell Dev Biol* **14**: 399-458.

Green, C.B., Cheng, G., Chandra, J., Mukherjee, P., Ghannoum, M.A., and Hoyer, L.L. (2004) RT-PCR detection of *Candida albicans ALS* gene expression in the reconstituted human epithelium (RHE) model of oral candidiasis and in model biofilms. *Microbiology* **150**: 267-275.

Green, R., and Noller, H.F. (1997) Ribosomes and translation. *Annu Rev Biochem* **66**: 679-716.

Grewal, S.I., and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* **301**: 798-802.

Grosschedl, R., Giese, K., and Pagel, J. (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. *Trends Genet* **10**: 94-100.

Guo, B., Styles, C.A., Feng, Q., and Fink, G.R. (2000) A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc Natl Acad Sci U S A* **97**: 12158-12163.

Gustin, M.C., Albertyn, J., Alexander, M., and Davenport, K. (1998) MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* **62**: 1264-1300.

Halme, A., Bumgarner, S., Styles, C., and Fink, G.R. (2004) Genetic and epigenetic regulation of the *FLO* gene family generates cell-surface variation in yeast. *Cell* **116**: 405-415.

Hazen, K.C., Brawner, D.L., Riesselman, M.H., Jutila, M.A., and Cutler, J.E. (1991) Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. *Infect Immun* **59**: 907-912.

Heard, E., Clerc, P., and Avner, P. (1997) X-chromosome inactivation in mammals. *Annu Rev Genet* **31**: 571-610.

Herskowitz, I. (1989) A regulatory hierarchy for cell specialization in yeast. *Nature* **342**: 749-757.

Hinnebusch, A.G. (1984) Evidence for translational regulation of the activator of general amino acid control in yeast. *Proc Natl Acad Sci U S A* **81**: 6442-6446.

Hinnebusch, A.G. (1994) Translational control of *GCN4*: an in vivo barometer of initiation-factor activity. *Trends Biochem Sci* **19**: 409-414.

Hinnebusch, A.G. (1997) Translational regulation of yeast *GCN4*. A window on factors that control initiator-tRNA binding to the ribosome. *J Biol Chem* **272**: 21661-21664.

Hinnebusch, A.G., and Natarajan, K. (2002) Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress. *Eukaryot Cell* **1**: 22-32.

Hong, L., Schroth, G.P., Matthews, H.R., Yau, P., and Bradbury, E.M. (1993) Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 "tail" to DNA. *J Biol Chem* **268**: 305-314.

Hoyer, L.L. (2001) The *ALS* gene family of *Candida albicans*. *Trends Microbiol* **9**: 176-180.

Huang, Y. (2002) Transcriptional silencing in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Nucleic Acids Res* **30**: 1465-1482.

Jackson, J.D., Falciano, V.T., and Gorovsky, M.A. (1996) A likely histone H2A.F/Z variant in *Saccharomyces cerevisiae*. *Trends Biochem Sci* **21**: 466-467.

Johnson, D.I. (1999) Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiol Mol Biol Rev* **63**: 54-105.

Khan, A.U., and Krishnamurthy, S. (2005) Histone modifications as key regulators of transcription. *Front Biosci* **10**: 866-872.

Khochbin, S., Verdel, A., Lemercier, C., and Seigneurin-Berny, D. (2001) Functional significance of histone deacetylase diversity. *Curr Opin Genet Dev* **11**: 162-166.

Kleinschmidt, M., Grundmann, O., Blüthgen, N., Mösch, H.U., and Braus, G.H. (2005) Transcriptional profiling of *Saccharomyces cerevisiae* cells under adhesion-inducing conditions. *Mol Genet Genomics* **273**: 382-393.

Kobayashi, O., Hayashi, N., Kuroki, R., and Sone, H. (1998) Region of FLO1 proteins responsible for sugar recognition. *J Bacteriol* **180**: 6503-6510.

Krebs, J.E., Kuo, M.H., Allis, C.D., and Peterson, C.L. (1999) Cell cycle-regulated histone acetylation required for expression of the yeast *HO* gene. *Genes Dev* **13**: 1412-1421.

Kronstad, J., De Maria, A.D., Funnell, D., Laidlaw, R.D., Lee, N., de Sa, M.M., and Ramesh, M. (1998) Signaling via cAMP in fungi: interconnections with mitogen-activated protein kinase pathways. *Arch Microbiol* **170**: 395-404.

Kuchin, S., Vyas, V.K., and Carlson, M. (2002) Snf1 protein kinase and the repressors Nrg1 and Nrg2 regulate *FLO11*, haploid invasive growth, and diploid pseudohyphal differentiation. *Mol Cell Biol* **22**: 3994-4000.

Kuo, M.H., vom Baur, E., Struhl, K., and Allis, C.D. (2000) Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol Cell* **6**: 1309-1320.

Lambrechts, M.G., Bauer, F.F., Marmur, J., and Pretorius, I.S. (1996) Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. *Proc Natl Acad Sci U S A* **93**: 8419-8424.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., Zeitlinger, J., Jennings, E.G., Murray, H.L., Gordon, D.B., Ren, B., Wyrick, J.J., Tagne, J.B., Volkert, T.L., Fraenkel, E., Gifford, D.K., and Young, R.A. (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* **298**: 799-804.

Lengeler, K.B., Davidson, R.C., D'Souza, C., Harashima, T., Shen, W.C., Wang, P., Pan, X., Waugh, M., and Heitman, J. (2000) Signal transduction cascades regulating fungal development and virulence. *Microbiol Mol Biol Rev* **64**: 746-785.

Lewis, B.A., and Reinberg, D. (2003) The mediator coactivator complex: functional and physical roles in transcriptional regulation. *J Cell Sci* **116**: 3667-3675.

Li, F., and Palecek, S.P. (2003) EAP1, a *Candida albicans* gene involved in binding human epithelial cells. *Eukaryot Cell* **2**: 1266-1273.

Liu, H., Styles, C.A., and Fink, G.R. (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* **262**: 1741-1744.

Lo, W.S., and Dranginis, A.M. (1996) *FLO11*, a yeast gene related to the *STA* genes, encodes a novel cell surface flocculin. *J Bacteriol* **178**: 7144-7151.

Lo, W.S., and Dranginis, A.M. (1998) The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol Biol Cell* **9**: 161-171.

Loeb, J.D., Kerentseva, T.A., Pan, T., Sepulveda-Becerra, M., and Liu, H. (1999) *Saccharomyces cerevisiae* G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway. *Genetics* **153**: 1535-1546.

Lorch, Y., Zhang, M., and Kornberg, R.D. (1999) Histone octamer transfer by a chromatin-remodeling complex. *Cell* **96**: 389-392.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**: 251-260.

Luger, K., and Richmond, T.J. (1998) DNA binding within the nucleosome core. *Curr Opin Struct Biol* **8**: 33-40.

Madhani, H.D., and Fink, G.R. (1997) Combinatorial control required for the specificity of yeast MAPK signaling. *Science* **275**: 1314-1317.

Mager, W.H., and Planta, R.J. (1990) Multifunctional DNA-binding proteins mediate concerted transcription activation of yeast ribosomal protein genes. *Biochim Biophys Acta* **1050**: 351-355.

Meersseman, G., Pennings, S., and Bradbury, E.M. (1992) Mobile nucleosomes--a general behavior. *EMBO J* **11**: 2951-2959.

Meneghini, M.D., Wu, M., and Madhani, H.D. (2003) Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**: 725-736.

Mohrmann, L., and Verrijzer, C.P. (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim Biophys Acta* **1681**: 59-73.

Mösch, H.U., Roberts, R.L., and Fink, G.R. (1996) Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* **93**: 5352-5356.

Mösch, H.U. (2000) Pseudohyphal development of *Saccharomyces cerevisiae*. *Contrib Microbiol* **5**: 185-200.

Natarajan, K., Meyer, M.R., Jackson, B.M., Slade, D., Roberts, C., Hinnebusch, A.G., and Marton, M.J. (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol* **21**: 4347-4368.

Oehlen, L.J., and Cross, F.R. (1998) Potential regulation of Ste20 function by the Cln1-Cdc28 and Cln2-Cdc28 cyclin-dependent protein kinases. *J Biol Chem* **273**: 25089-25097.

Palecek, S.P., Parikh, A.S., and Kron, S.J. (2002) Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth. *Microbiology* **148**: 893-907.

Pan, X., and Heitman, J. (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Mol Cell Biol* **19**: 4874-4887.

Pan, X., Harashima, T., and Heitman, J. (2000) Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Curr Opin Microbiol* **3**: 567-572.

Planta, R.J., and Mager, W.H. (1998) The list of cytoplasmic ribosomal proteins of *Saccharomyces cerevisiae*. *Yeast* **14**: 471-477.

Pollard, K.J., and Peterson, C.L. (1997) Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. *Mol Cell Biol* **17**: 6212-6222.

Posas, F., Takekawa, M., and Saito, H. (1998) Signal transduction by MAP kinase cascades in budding yeast. *Curr Opin Microbiol* **1**: 175-182.

Proweller, A., and Butler, S. (1994) Efficient translation of poly(A)-deficient mRNAs in *Saccharomyces cerevisiae*. *Genes Dev* **8**: 2629-2640.

Raue, H.A., Mager, W.H., and Planta, R.J. (1991) Structural and functional analysis of yeast ribosomal proteins. *Methods Enzymol* **194**: 453-477.

Reeve, J.N., Bailey, K.A., Li, W.T., Marc, F., Sandman, K., and Soares, D.J. (2004) Archaeal histones: structures, stability and DNA binding. *Biochem Soc Trans* **32**: 227-230.

Reid, J.L., Iyer, V.R., Brown, P.O., and Struhl, K. (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell* **6**: 1297-1307.

Reinberg, D., Orphanides, G., Ebright, R., Akoulitchev, S., Carcamo, J., Cho, H., Cortes, P., Drapkin, R., Flores, O., Ha, I., Inostroza, J.A., Kim, S., Kim, T.K., Kumar, P., Lagrange, T., LeRoy, G., Lu, H., Ma, D.M., Maldonado, E., Merino, A., Mermelstein, F., Olave, I., Sheldon, M., Shiekhattar, R., Zawel, L., and et al. (1998) The RNA polymerase II general transcription factors: past, present, and future. *Cold Spring Harb Symp Quant Biol* **63**: 83-103.

Reynolds, T.B., and Fink, G.R. (2001) Bakers' yeast, a model for fungal biofilm formation. *Science* **291**: 878-881.

Rhode, P.R., Elsasser, S., and Campbell, J.L. (1992) Role of multifunctional autonomously replicating sequence binding factor 1 in the initiation of DNA replication and transcriptional control in *Saccharomyces cerevisiae*. *Mol Cell Biol* **12**: 1064-1077.

Roberts, R.L., and Fink, G.R. (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev* **8**: 2974-2985.

Robertson, L.S., and Fink, G.R. (1998) The three yeast A kinases have specific signaling functions in pseudohyphal growth. *Proc Natl Acad Sci U S A* **95**: 13783-13787.

Robertson, L.S., Causton, H.C., Young, R.A., and Fink, G.R. (2000) The yeast A kinases differentially regulate iron uptake and respiratory function. *Proc Natl Acad Sci U S A* **97**: 5984-5988.

Roeder, R.G. (1998) Role of general and gene-specific cofactors in the regulation of eukaryotic transcription. *Cold Spring Harb Symp Quant Biol* **63**: 201-218.

Rupp, S., Summers, E., Lo, H.J., Madhani, H., and Fink, G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J* **18**: 1257-1269.

Sanchez-Martinez, C., and Perez-Martin, J. (2001) Dimorphism in fungal pathogens: *Candida albicans* and *Ustilago maydis*--similar inputs, different outputs. *Curr Opin Microbiol* **4**: 214-221.

Sandman, K., and Reeve, J.N. (2000) Structure and functional relationships of archaeal and eukaryal histones and nucleosomes. *Arch Microbiol* **173**: 165-169.

Santisteban, M.S., Kalashnikova, T., and Smith, M.M. (2000) Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. *Cell* **103**: 411-422.

Schultz, M.C., Reeder, R.H., and Hahn, S. (1992) Variants of the TATA-binding protein can distinguish subsets of RNA polymerase I, II, and III promoters. *Cell* **69**: 697-702.

Searfoss, A.M., and Wickner, R.B. (2000) 3' poly(A) is dispensable for translation. *Proc Natl Acad Sci U S A* **97**: 9133-9137.

Sif, S. (2004) ATP-dependent nucleosome remodeling complexes: enzymes tailored to deal with chromatin. *J Cell Biochem* **91**: 1087-1098.

Slayter, H.S., Warner, J.R., Rich, A., and Hall, C.E. (1963) The Visualization of Polyribosomal Structure. *J Mol Biol* **17**: 652-657.

Spahn, C.M., Beckmann, R., Eswar, N., Penczek, P.A., Sali, A., Blobel, G., and Frank, J. (2001) Structure of the 80S ribosome from *Saccharomyces cerevisiae*--tRNA-ribosome and subunit-subunit interactions. *Cell* **107**: 373-386.

Stargell, L.A., Moqtaderi, Z., Dorris, D.R., Ogg, R.C., and Struhl, K. (2000) TFIIA has activator-dependent and core promoter functions *in vivo*. *J Biol Chem* **275**: 12374-12380.

Steger, D.J., and Workman, J.L. (1996) Remodeling chromatin structures for transcription: what happens to the histones? *Bioessays* **18**: 875-884.

Stewart, J.J., and Stargell, L.A. (2001) The stability of the TFIIA-TBP-DNA complex is dependent on the sequence of the TATAAA element. *J Biol Chem* **276**: 30078-30084.

Struhl, K., Chen, W., Hill, D.E., Hope, I.A., and Oettinger, M.A. (1985) Constitutive and coordinately regulated transcription of yeast genes: promoter elements, positive and negative regulatory sites, and DNA binding proteins. *Cold Spring Harb Symp Quant Biol* **50**: 489-503.

Struhl, K. (1995) Yeast transcriptional regulatory mechanisms. *Annu Rev Genet* **29**: 651-674.

Struhl, K. (1997) Selective roles for TATA-binding-protein-associated factors *in vivo*. *Genes Funct* **1**: 5-9.

Suto, R.K., Clarkson, M.J., Tremethick, D.J., and Luger, K. (2000) Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat Struct Biol* **7**: 1121-1124.

Taheri, N., Köhler, T., Braus, G.H., and Mösch, H.U. (2000) Asymmetrically localized Bud8p and Bud9p proteins control yeast cell polarity and development. *EMBO J* **19**: 6686-6696.

Teunissen, A.W., and Steensma, H.Y. (1995) Review: the dominant flocculation genes of *Saccharomyces cerevisiae* constitute a new subtelomeric gene family. *Yeast* **11**: 1001-1013.

Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J., and Wu, C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev* **13**: 686-697.

Ullmann, A., Jacob, F., and Monod, J. (1967) Characterization by in vitro complementation of a peptide corresponding to an operator-proximal segment of the beta-galactosidase structural gene of *Escherichia coli*. *J Mol Biol* **24**: 339-343.

Valente, L., and Kinzy, T.G. (2003) Yeast as a sensor of factors affecting the accuracy of protein synthesis. *Cell Mol Life Sci* **60**: 2115-2130.

van Dyk, D., Hansson, G., Pretorius, I.S., and Bauer, F.F. (2003) Cellular differentiation in response to nutrient availability: The repressor of meiosis, Rme1p, positively regulates invasive growth in *Saccharomyces cerevisiae*. *Genetics* **165**: 1045-1058.

van Dyk, D., Pretorius, I.S., and Bauer, F.F. (2005) Mss11p is a central element of the regulatory network that controls *FLO11* expression and invasive growth in *Saccharomyces cerevisiae*. *Genetics* **169**: 91-106.

Velculescu, V.E., Zhang, L., Zhou, W., Vogelstein, J., Basrai, M.A., Bassett, D.E., Jr., Hieter, P., Vogelstein, B., and Kinzler, K.W. (1997) Characterization of the yeast transcriptome. *Cell* **88**: 243-251.

Verschoor, A., Warner, J.R., Srivastava, S., Grassucci, R.A., and Frank, J. (1998) Three-dimensional structure of the yeast ribosome. *Nucleic Acids Res* **26**: 655-661.

Verstrepen, K.J., Reynolds, T.B., and Fink, G.R. (2004) Origins of variation in the fungal cell surface. *Nat Rev Microbiol* **2**: 533-540.

Vilela, C., and McCarthy, J.E. (2003) Regulation of fungal gene expression via short open reading frames in the mRNA 5'untranslated region. *Mol Microbiol* **49**: 859-867.

Vogelauer, M., Wu, J., Suka, N., and Grunstein, M. (2000) Global histone acetylation and deacetylation in yeast. *Nature* **408**: 495-498.

Wallrath, L.L., Lu, Q., Granok, H., and Elgin, S.C. (1994) Architectural variations of inducible eukaryotic promoters: preset and remodeling chromatin structures. *Bioessays* **16**: 165-170.

Wallrath, L.L. (1998) Unfolding the mysteries of heterochromatin. *Curr Opin Genet Dev* **8**: 147-153.

Ward, M.P., Gimeno, C.J., Fink, G.R., and Garrett, S. (1995) SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Mol Cell Biol* **15**: 6854-6863.

Weber, J., Jelinek, W., and Darnell, J.E., Jr. (1977) The definition of a large viral transcription unit late in Ad2 infection of HeLa cells: mapping of nascent RNA molecules labeled in isolated nuclei. *Cell* **10**: 611-616.

Weiss, K., and Simpson, R.T. (1998) High-resolution structural analysis of chromatin at specific loci: *Saccharomyces cerevisiae* silent mating type locus *HML*alpha. *Mol Cell Biol* **18**: 5392-5403.

Wek, S.A., Zhu, S., and Wek, R.C. (1995) The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. *Mol Cell Biol* **15**: 4497-4506.

Wimberly, B.T., Brodersen, D.E., Clemons, W.M., Jr., Morgan-Warren, R.J., Carter, A.P., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. (2000) Structure of the 30S ribosomal subunit. *Nature* **407**: 327-339.

Winston, F., and Carlson, M. (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet* **8**: 387-391.

Wolffe, A.P. (1995) Chromatin. Structure and function. *Academic Press, London*.

Workman, J.L., and Roeder, R.G. (1987) Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell* **51**: 613-622.

Yatherajam, G., Zhang, L., Kraemer, S.M., and Stargell, L.A. (2003) Protein-protein interaction map for yeast TFIID. *Nucleic Acids Res* **31**: 1252-1260.

Yuan, G.C., Liu, Y.J., Dion, M.F., Slack, M.D., Wu, L.F., Altschuler, S.J., and Rando, O.J. (2005) Genome-scale identification of nucleosome positions in *S. cerevisiae*. *Science* **309**: 626-630.

Yun, D.F., Laz, T.M., Clements, J.M., and Sherman, F. (1996) mRNA sequences influencing translation and the selection of AUG initiator codons in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* **19**: 1225-1239.

Zorio, D.A., and Bentley, D.L. (2004) The link between mRNA processing and transcription: communication works both ways. *Exp Cell Res* **296**: 91-97.

Chapter 2

Chromatin remodeling factors and the histone variant H2A.Z/Htz1p counteract in regulating *FLO11* expression in *Saccharomyces cerevisiae*

Abstract

The *FLO11* gene encoding a cell-surface adhesin is strongly repressed by one of the largest promoters of the genome when diploid *Saccharomyces cerevisiae* grows in its non-adherent yeast form. The chromatin structure of the 3.7 kb DNA region between the start codons of the adjacent and divergently transcribed *FLO11* and *MRS1* genes was mapped. The restrained *FLO11* promoter comprises 3.0 kb of protective chromatin which is only interrupted by a single MNase-sensitive site located 1.2 kb upstream of the *FLO11* open reading frame. This site coincides with the Sfl1p repressor binding site. Besides repression of *FLO11* by Sfl1p, the histone H2A variant Htz1p was found to be required to keep *FLO11* in a silent state. The chromatin remodeler Rsc1p is not required for *FLO11* repression but for induction resulting in adhesive filamentous growth of diploid and haploid cells. The histone acetyl transferase Gcn5p is also required for diploid and haploid adhesive growth. We suggest that the yeast cell is able to prevent *FLO11* expression by a combination of repressor and chromatin dependent silencing. Induction of *FLO11* and subsequently adherence and filamentous growth are achieved by multiple distinct and chromatin dependent transcriptional mechanisms.

Introduction

Specific regulation of gene expression is essential for cells and organisms during development and as response to environmental signals. This includes both inactivation and activation of transcription of specific genes.

Eukaryotes evolved multiple mechanisms on different levels to inhibit or activate gene expression in cells. Besides the control of transcriptional initiation and processing, post-transcriptional regulatory processes affecting mRNA stability, mRNA translation, protein targeting or stability have to be considered (Gray and Wickens, 1998). Gene repression on the level of DNA signal sequences is usually mediated by DNA binding proteins which act directly or in combination with corepressors as e.g. the Tup1p-Ssn6p complex that has originally been identified in yeast. Histones are additional DNA binding proteins, which determine the local chromatin structure and hence have profound effects on transcriptional activity (Grewal and Moazed, 2003; Horn and Peterson, 2002; Narlikar *et al.*, 2002). The chromatin of chromosomal DNA can be divided into active euchromatin and repressive heterochromatin. The dense structure of the heterochromatin inhibits the binding of DNA-binding proteins to DNA, excluding the transcription machinery from genes of those regions, thereby, giving rise to silent genes (Grewal and Moazed, 2003).

The local arrangements of the nucleosomal structure and chromatin in general are regulated in many respects. Besides the regular histones, there are histone variants, which can be incorporated into chromatin as e.g. the histone H2A variant H2A.Z, which is encoded by the *HTZ1* gene in yeast. Htz1p seems not only to play an important role in transcriptional regulation, but as well for the function of entire chromosomes (Adam *et al.*, 2001; Krogan *et al.*, 2003; Larochelle and Gaudreau, 2003; Santisteban *et al.*, 2000; Smith and Johnson, 2000). H2A.Z/Htz1p is preferentially incorporated into intergenic regions and seems to have partially redundant functions with nucleosome remodeling complexes by modulating functional interactions to regulatory components of transcription. Htz1p is also

required for centromer function and accurate chromosome segregation (Rangasamy *et al.*, 2004) and protects euchromatin from the ectopic spread of silent heterochromatin (Meneghini *et al.*, 2003).

Histones can be modified by covalent acetylation of lysine residues, methylation of arginine and lysine residues, phosphorylation of serine residues, or ubiquitination of lysine residues (Iizuka and Smith, 2003). The chromatin structure is highly dynamic and requires these modifications to control gene expression (Iizuka and Smith, 2003). Acetylation plays a prominent role in histone modification with SAGA, Ada, and SLIK as large complexes, which all possess Gcn5p as functional histone acetyl transferase (Balasubramanian *et al.*, 2002; Pray-Grant *et al.*, 2002; Wu *et al.*, 2004). Transcriptome data revealed that Gcn5p is involved in the regulation of numerous genes with overlapping functions to the histone acetylase of TFIID (Lee *et al.*, 2000). Histone acetylation and ATP-dependent chromatin remodeling often are coupled processes in gene regulation (Neely and Workman, 2002).

Additional large multisubunit complexes (e.g. ISWI, Swi/Snf, or the Srb/mediator/holoenzyme complex as central part of the transcriptional machinery) are required to slide or to remodel nucleosomes (Narlikar *et al.*, 2002; Perez-Martin, 1999). The Isw2p/Itc1p complex, a member of the ISWI remodeler family, has been described to be involved in the transcriptional repression and derepression of a broad spectrum of genes (Fazzio *et al.*, 2001). They include the regulation of starvation responses, developmental processes (Kent *et al.*, 2001), or the repression of cell-type specific genes of Mata yeast cells (Ruiz *et al.*, 2003). The complex modulates the accessibility of the transcriptional machinery to chromatin and presumably functions by a nucleosome sliding mechanism (Fazzio and Tsukiyama, 2003). RSC (for remodels the structure of chromatin) is another ATP-dependent complex of the Snf2p/Swi2p family that is essential for viability and cell cycle progression (Angus-Hill *et al.*, 2001; Cairns *et al.*, 1996; Du *et al.*, 1998). Two biochemically distinct forms of the RSC complex have been identified, one with Rsc1p as a member, the second with Rsc2p (Cairns *et al.*, 1999). Both complexes exhibit distinct functions but also share partially redundant functions (Bungard *et al.*, 2004; Yukawa *et al.*, 2002).

Dimorphic yeasts vary the expression of special genes resulting in either a single cell growth mode (yeast form) or in connected cells resulting in multicellular filaments. The *Saccharomyces cerevisiae FLO11* gene encodes a glycosyl-phosphatidylinositol (GPI)-linked cell surface protein, which has to be expressed for cell-cell and cell-surface adhesion. Therefore, *FLO11* activation is the prerequisite for invasive growth of haploid cells as well as pseudohyphal development of diploids. Repression of *FLO11* is necessary for the growth as single yeast cells of *S. cerevisiae*. In diploid yeast cells growing in the yeast form, *FLO11* expression is strongly repressed and its mRNA is hardly detectable (Braus *et al.*, 2003; Gimeno *et al.*, 1992; Lambrechts *et al.*, 1996; Lo and Dranginis, 1998).

Dimorphism of the baker's yeast *S. cerevisiae*, and therefore, preventing or activating gene expression is dependent on nutrient supply (Braus *et al.*, 2003; Gimeno *et al.*, 1992; Lambrechts *et al.*, 1996; Lo and Dranginis, 1998). Starvation of glucose causes the switch from loosely associated haploid yeast cells to invasive growth. This switch is the result of the activation of the adhesin encoding *FLO11* gene, which in haploid cells is *per se* partially derepressed even without nutrient limitation. This partial derepression of *FLO11*, however, is not sufficient to cause adhesive growth. In diploid *S. cerevisiae* cells, however, *FLO11* is strongly repressed when cells grow as single non-adhesive yeast cells in the absence of nutrient limitation. Starvation for nitrogen overcomes this inhibition, and induces *FLO11* expression and subsequently leads to pseudohyphal growth with elongated cells connected by the adhesin (Gancedo, 2001; Pan *et al.*, 2000).

Further well known regulators of the *FLO11* gene expression are the final transcription factors Tec1p and Flo8p at the end of the MAPK cascade and the cAMP pathway, respectively (Pan and Heitman, 1999; Rupp *et al.*, 1999).

In this work, we examined the impact of chromatin factors on repression and derepression of the *FLO11* gene. We investigated haploid and diploid *FLO11* transcription and the resulting growth form under non-limiting repressive and glucose limiting derepressive conditions. Furthermore, we analysed the effect of overexpressed transcription factors Tec1p and Tpk2p on adhesive growth and *FLO11* transcription. Our results show various chromatin organisation activities including

nucleosome architecture, histone modifcation and nucleosome remodeling, which determine the level of *FLO11* expression.

Materials and Methods

Yeast strains and growth conditions

All yeast strains used in this study and their genotypes are listed in Table 1. All strains are derivatives of the *S. cerevisiae* Σ1278b genetic strain background (Gimeno *et al.*, 1992). Deletion mutants were obtained by amplifying the respective deletion cassette, carrying the kan^R marker gene, of the respective BY strain (Brachmann *et al.*, 1998). Transformations were carried out using the lithium-acetate yeast transformation method (Ito *et al.*, 1983). The integrations were confirmed by Southern blot analysis (Southern, 1975) or PCR (Saiki *et al.*, 1985). Haploid strains were crossed to produce diploid mutant strains.

Strains were generally cultivated in YPD complete medium (1 % yeast extract, 2 % bacto peptone, 2 % glucose) at 30°C. For selection of gene disruption 200 µg/ml geneticin G418 was added. For wash tests, cells were grown on solid YPD medium (2 % agar), alternatively on synthetic minimal medium (YNB; 0.15 % Yeast Nitrogen Base, 50 mM $(NH_4)_2SO_4$, 200 mM myo-inositol, 20 % glucose) supplemented with the appropriate amino acids. Low ammonium medium (SLAD; YNB medium containing 50 µM ammonium sulfate) was used to observe pseudohyphal formation.

Plasmids

Plasmids used in this study are listed in Table 2. Deletion cassettes were amplified from the respective Euroscarf deletion strain (Brachmann *et al.*, 1998), and inserted via TA-cloning in pBlueskript II SK (Stratagene) using the restriction site *Eco*RV.

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
RH2816	*MAT*α, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *HIS* $^+$	Our collection
RH2817	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *HIS* $^+$	Our collection
RH2818	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *HIS* $^+$	Our collection
RH2819	*MAT*a, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *HIS* $^+$	Our collection
RH2656	*MATa/α, ura3-52*/*ura3-52*, *trp1*::*hisG*/*TRP1*	Braus *et al*., 2003
RH2661	*MATa/α, ura3-52*/*ura3-52*, *trp1*::*hisG*/*TRP1*, *Δflo11*::*kan*^{R}/*Δflo11*::*kan*^{R}	Braus *et al*., 2003
RH2662	*MAT*a, *ura3-52*, *Δflo11*::*kan*^{R}	Braus *et al*., 2003
RH3184	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δrsc1*::*kan*^{R}, *HIS* $^+$	This study
RH3185	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δrsc1*::*kan*^{R}, *HIS* $^+$	This study
RH3186	*MATa/α, ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2* *Δrsc1*::*kan*^{R}/*Δrsc1*::*kan*^{R}, *HIS*$^+$	This study
RH3187	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δisw2*::*kan*^{R} , *HIS* $^+$	This study
RH3188	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δisw2*::*kan*^{R}, *HIS* $^+$	This study
RH3189	*MATa/α, ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δisw2*::*kan*^{R}/*Δisw2*::*kan*^{R}, *HIS* $^+$	This study
RH3190	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3191	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3192	*MATa/α, ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δgcn5*::*kan*^{R}/*Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3214	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3215	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3216	*MATa/α, ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δhtz1*::*kan*^{R}/*Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3217	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δssn6*::*kan*^{R}, *HIS* $^+$	This study
RH3218	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δssn6*::*kan*^{R}, *HIS* $^+$	This study

Table 1. continued

Strain	Genotype	Source
RH3219	*MATa/α, ura3-52/ura3-52, his3::hisG/his3::hisG, trp1::hisG/TRP1, leu2::hisG/LEU2, Δssn6::kan^R/Δssn6::kan^R, HIS$^+$*	This study
RH3220	*MATα, ura3-52, his3::hisG, trp1::hisG, Δsfl1::kan^R, HIS$^+$*	This study
RH3221	*MAT*a, *ura3-52, his3::hisG, leu2::hisG, Δsfl1::kan^R, HIS$^+$*	This study
RH3222	*MATa/α, ura3-52/ura3-52, his3::hisG/his3::hisG, trp1::hisG/TRP1, leu2::hisG/LEU2, Δsfl1::kan^R/Δsfl1::kan^R, HIS$^+$*	This study
RH3267	*MATα, ura3-52, his3::hisG, trp1::hisG, Δrsc1::NAT, Δsfl1:: kan^R, HIS$^+$*	This study
RH3268	*MAT*a, *ura3-52, his3::hisG, trp1::hisG, Δrsc1::NAT, Δsfl1:: kan^R, HIS$^+$*	This study

Genetic crosses, sporulation, and tetrad dissection

Diploid homozygous *S. cerevisiae* strains were obtained by mating of haploid yeast strains that were verified by Southern hybridization experiments. Mating, sporulation and tetrad dissection were performed according to Sherman (Sherman, 1991).

Adhesive growth and pseudohyphal formation assays

Adhesive growth of haploids and diploids were performed as described previously (Roberts and Fink, 1994). Cells were patched on the respective medium and incubated for one or five days at 30°C. Plates were photographed to secure total growth and washed under a stream of water. The remaining adhesive cells were documented by photographing the washed plate.

Pseudohyphal growth after growing five days on SLAD medium at 30°C was viewed using an Axiovert microscope (Carl Zeiss, Jena, Germany), and photographed with a

Kappa DX30 digital camera and the Kappa Image Base software (Kappa Opta-Electronics, Gleichen, Germany).

Table 2. Plasmids used in this study.

Plasmid	Description	Source
pBIISK(+)	Commercial cloning vector	Stratagene, La Jolla, USA
pRS316	*URA3*-marked centromere vector	Sikorski and Hieter, 1989
pME2071	2.5 kb *GAL1(p)*::*TEC1* fusion in pRS316	Mösch *et al.*, 1999
pME2632	pBIISK(+) with integrated deletion cassette of *RSC1* ORF (*kan*^{R})	This study
pME2626	pBIISK(+) with integrated deletion cassette of *ISW2* ORF (*kan*^{R})	This study
pME2628	pBIISK(+) with integrated deletion cassette of *GCN5* ORF (*kan*^{R})	This study
pME2631	pBIISK(+) with integrated deletion cassette of *HTZ1* ORF (*kan*^{R})	This study
pME2648	pBIISK(+) with integrated deletion cassette of *SSN6* ORF (*kan*^{R})	This study
pME2653	pBIISK(+) with integrated deletion cassette of *SFL1* ORF (*kan*^{R})	This study

Genomic structure analysis

Chromatin extraction and MNase treatment were performed as described previously (Thoma, 1996). For indirect end-labelling, the chromosomal MNase treated DNA was digested with *Eco*RI and *Bam*HI or *Dra*I, respectively, and fractionated on 1.2 % agarose gels. The DNA was transferred by the alkaline blotting method to a Hybond-N nylon membrane and hybridized to appropriate radioactively labelled probes. A DNA ladder consisting of multiples of 256 bp was used for calibration (Thoma *et al.*, 1984).

RNA analyis

Total RNAs were isolated from exponentially growing yeast cells according to the method of Cross and Tinkelenberg (Cross and Tinkelenberg, 1991). For Northern hybridization analysis, 20 μg of total RNAs were separated on a 1.4 % agarose gel containing 3 % formaldehyde, and transferred onto nylon membranes by capillary blotting. Transcripts of *FLO11* and *ACT1* were detected by using specific ^{32}P-radiolabeled DNA fragments with the Prime-It labelling kit from Stratagene (La Jolla, USA). Signal intensities were visualized and quantified using a BAS-1500 phosphorimaging scanner (Fuji, Tokyo, Japan).

Results

The silenced *FLO11* promoter in yeast shows a single *Micrococcus* nuclease sensitive region comprising the Sfl1p repressor binding site

Chromatin and activities involved in its establishment, maintenance or remodeling play a pivotal role in gene silencing and expression. The ability of yeast cells to grow adhesively on solid surfaces under certain circumstances largely depends on the regulated derepression of otherwise silent *FLO* genes. In Σ1278b strains, *FLO11* is the key player of this tightly controlled adhesion phenotype. Pseudohyphal development in diploid Σ-strains essentially takes place upon nitrogen limitation, and also depends on controlled *FLO11* derepression.

In first experiments, we tempted to resolve the *FLO11* promoter chromatin structure by *Micrococcus* nuclease (MNase) protecting experiments for the repressed and derepressed *FLO11* gene. Derepression of *FLO11*, however, causes severe cell wall changes (Kleinschmidt *et al.*, 2005) making the isolation of high quality chromatin impossible (data not shown). Therefore, we started by analysing the nucleosomal structure of the silenced *FLO11* promoter under non-limiting growth conditions with MNase protecting experiments. Nuclear extracts of diploid yeast cells, cultivated in YNB, and therefore, in the single cell growth form, were isolated and partially digested with MNase (Thoma, 1996). MNase hydrolyses DNA of chromatin preferentially between two nucleosomes or in nucleosome-free regions. The generated DNA fragment patterns of partial MNase digests reflect the size of a single nucleosome (about 150 bp), and give rise to DNA fragments with multiples of that size. Here we used the *ACT1* gene as chromatin quality control, were nine nucleosomes (N1 to N9) are visible (Figure 1A). This DNA was subsequently used to map MNase-sensitive as well as MNase-resistant sites within the silent *FLO11* promoter. Only in one of five regions mapped (region I to V in Figure 1B/C bottom), a significant MNase-sensitive region appears that is positioned about 1.2 kb upstream of the translational ATG start codon in region III. This sensitive site is framed on

both sites by protecting nucleosomes (Figure 1C). Further upstream and downstream of that sensitive site, and similarly in regions II, IV, and V, chromatin patterns are similar to the digested control DNA, referring to MNase-resistant nucleosomal promoter DNA (data not shown for regions II, IV and V).

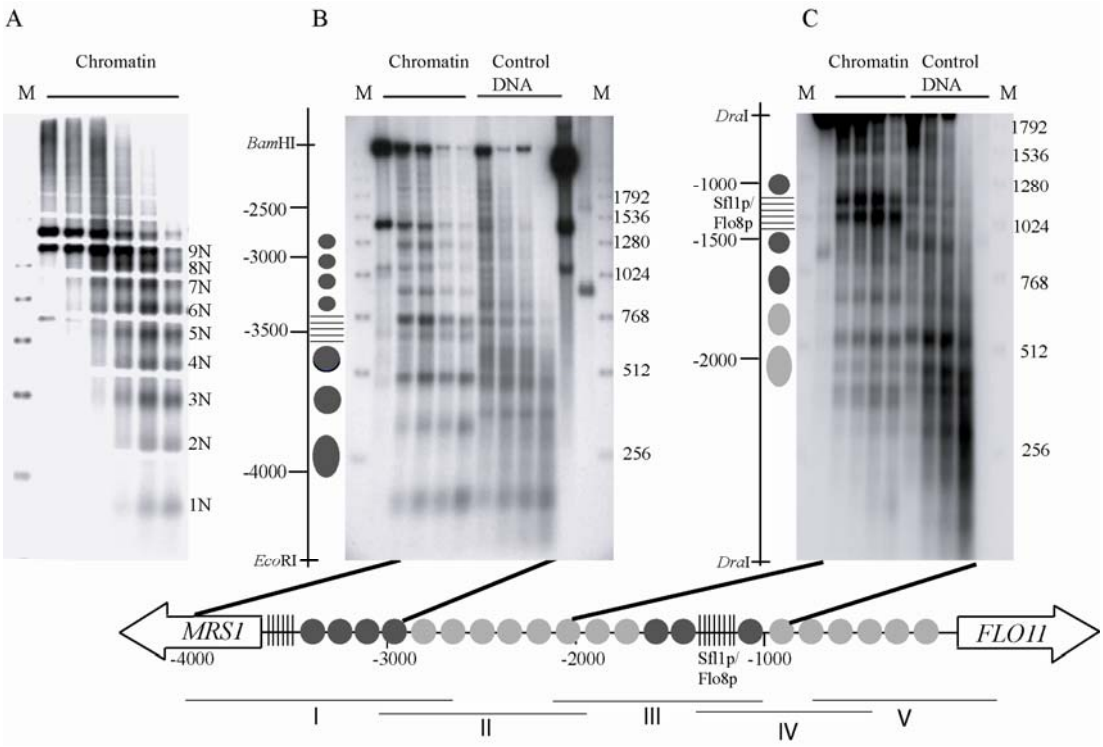

Figure 1. The chromatin structure of the silenced *FLO11* promoter of *S. cerevisiae* is sensitive to MNase at the Sfl1p repressor binding site. Diploid wildtype yeast cells (RH2656) were grown in minimal medium (YNB) to logarithmic phase and chromatin was isolated. **A.** Chromatin was digested with MNase and DNA fragments were hybridized with a ^{32}P-labeled *ACT1* riboprobe as quality control. A 256 bp-DNA ladder (M) was used as size marker. **B.** Isolated chromatin and naked control DNA were treated with MNase and subsequently digested with *Eco*RI and *Bam*HI. The 300 bp ^{32}P-labeled riboprobe downstream of the *Eco*RI restriction site of the *FLO11* promoter was used as probe for hybridization. The 256 bp-DNA ladder as size marker is indicated (M). Numbers indicate the positions upstream of the *FLO11* start codon. **C.** Chromatin and naked control DNA were digested with *Dra*I after MNase treatment. The ^{32}P-radiolabeled riboprobe used for hybridization comprises 350 bp downstream of the first restriction site of the *FLO11* promoter. Diagrams on the left and below depict the positions of nucleosomes. Sensitive sites to MNase are hatched. The intergenic region between the divergently transcribed genes *MRS1* and *FLO11*, which was mapped in the different experiments (I to V) is indicated below including nucleosome positions.

A second MNase-sensitive site located approximately 3.5 kb upstream of the *FLO11* start codon is presumably part of the divergently transcribed *MRS1* gene, which encodes a mitochondrial splicing factor (Kreike *et al.*, 1987). This second MNase-sensitive site is in close proximity (approximately 0.2 kb) to the *MRS1* start codon.
In summary, the *FLO11* promoter as part of the *MRS1-FLO11* intergenic region seems to be efficiently embedded in a 3 kb chromatin structure, which results in a silenced state of *FLO11* under the used conditions. This region is interrupted by only one sensitive site about 1.2 kb upstream of the *FLO11* start ATG. This region corresponds to the mapped binding site of the repressor protein Sfl1p (Conlan and Tzamarias, 2001), which is the antagonist of the transcriptional activator Flo8p that binds to the same region (Pan and Heitman, 2002) at inducing conditions. Therefore, the MNase sensitive site seems to be essential for a metastable state, in which the *FLO11* expression can be regulated via repressors or activators. These data suggest that besides repression by Sfl1p the tight promoter chromatin also plays a prominent role in suppressing *FLO11* activity.

The histone H2A variant H2A.Z/Htz1p encoding gene *HTZ1* is required for *FLO11* expression in diploid yeast cells

FLO11 transcription in diploid yeast cells (Σ1278b background) cultivated in rich medium (YPD) is efficiently repressed, which results in hardly detectable *FLO11* transcript levels. In haploid cells of the same genetic background (Σ1278b), however, significant steady state *FLO11* transcript levels are detectable at the same growth condition (Figure 2A). As shown before, a MNase sensitive region corresponds to the Sfl1p repressor binding site. The *SFL1* gene encodes a known repressor of *FLO11* (Conlan and Tzamarias, 2001), and its deletion resulted, as expected, in severely increased *FLO11* mRNA levels (Figure 2A). As consequence, and in contrast to wildtype cells, Δ*sfl1*/Δ*sfl1* cells grow hyper-adhesively after only one day of growth (Figure 2B).

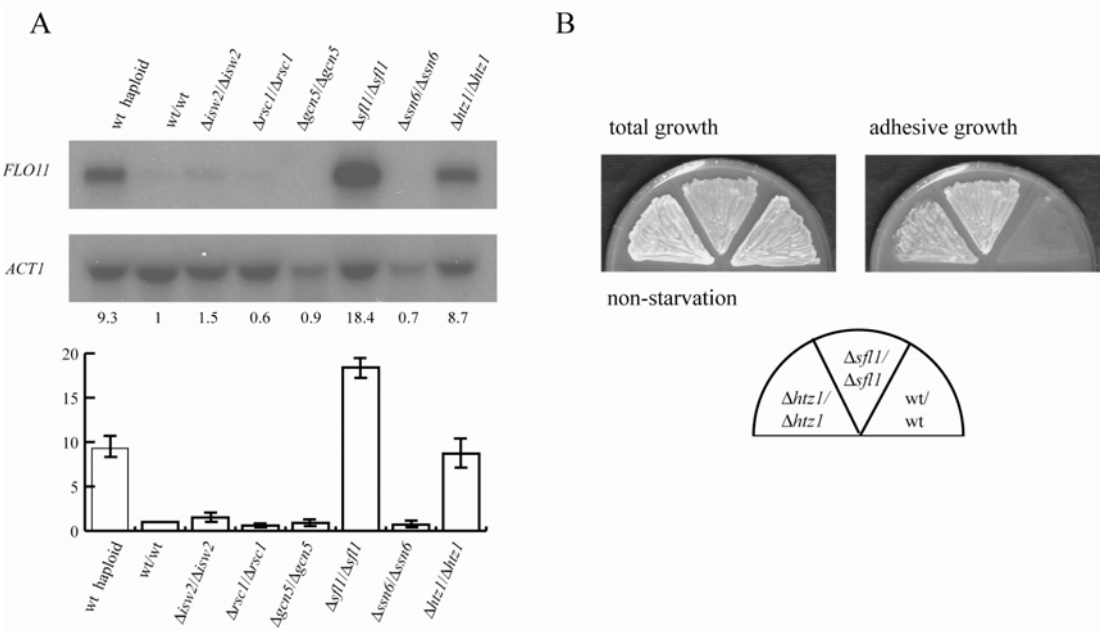

Figure 2. Diploid Δ*sfl1* and Δ*htz1* yeast cells exhibit increased *FLO11* mRNA levels and accelerated adhesive growth. A. Total RNAs were prepared from diploid yeast strains (Σ1278b) grown in complete YPD to logarithmic phase. Transcripts of diploid yeast strains carrying the homozygous mutation Δ*isw2*/Δ*isw2* (RH3189), Δ*rsc1*/Δ*rsc1* (RH3186), Δ*gcn5*/Δ*gcn5* (RH3192), Δ*ssn6*/Δ*ssn6* (RH3219), Δ*sfl1*/Δ*sfl1* (RH3222) and Δ*htz1*/Δ*htz1* (RH3216), respectively, were compared. A haploid wildtype (wt) strain (RH2817) and a diploid wt strain (RH2656) were used as control. 20 µg of total RNAs from each sample were subjected to Northern hybridization analyses for measurements of *FLO11* and *ACT1* transcript levels. Hybridization was performed using simultaneously ^{32}P-labeled riboprobes of *FLO11* and *ACT1*. Signals were quantified using a phosphorimaging scanner (Fuji Photofilm & Co. Ltd., Japan). Numbers given indicate relative expression levels of *FLO11* when compared to *ACT1* transcript as internal standard with a value for the diploid wt control strain corresponding to 1. **B.** *S. cerevisiae* strains with increased *FLO11* mRNA level were tested for accelerated adhesion. Δ*sfl1*/Δ*sfl1* (RH3222) and Δ*htz1*/Δ*htz1* (RH3216), and the wildtype control (RH2656) were plated on YPD and incubated for one day at 30°C when the wildtype as control has not yet developed an adhesive phenotype. Non-adhesive yeast cells were washed off the agar surface.

Although Tup1p-Ssn6p has been shown to be required for Sfl1p function in yeast cells of DBY747 background (Conlan and Tzamarias, 2001), in diploid Σ1278b cells

SSN6 does not seem to be involved in *FLO11* repression, as *FLO11* mRNA levels in Δ*ssn6*/Δ*ssn6* cells are similarly low as in wildtype cells (Figure 2A). In addition, deletion of neither *ISW2* (Δ*isw2*/Δ*isw2*) nor *RSC1* (Δ*rsc1*/Δ*rsc1*) does prevent efficient *FLO11* repression. Similarly, *GCN5*, which codes for the histone acetyl transferase (HAT) activity in complexes such as SAGA or Ada, is not required for *FLO11* repression (Figure 2A).

Deletion of the histone variant H2A.Z/Htz1p encoding gene *HTZ1*, however, relieves the silenced state of *FLO11* in diploid cells. Δ*htz1*/Δ*htz1* cells exhibit *FLO11* mRNA levels at least as high as haploid wildtype cells (Figure 2A), where *FLO11* expression is partially repressed. These data are further supported by the hyper-adhesiveness of Δ*htz1*/Δ*htz1* after only one day of growth on rich solid medium (Figure 2B), suggesting an activated metastable state of *FLO11*.

These data show that besides the known repressor Sfl1p also the histone H2A variant H2A.Z/Htz1p is an essential component for maintaining the silenced state of the *FLO11* gene and might be part of the protective chromatin structure of its promoter.

RSC1 and *GCN5* are required for *FLO11* dependent adhesion and pseudohyphal growth of diploid yeast cells

Diploid cells grow adhesively after five days of growth on solid rich medium as consequence of local glucose limitations, however, in their extent not as pronounced as glucose starved haploid cells do (Roberts and Fink, 1994). In Σ1278b cells, this adhesion is strictly *FLO11* dependent, and therefore, Δ*flo11*/Δ*flo11* cells are unable to adhere to the agar surface (Figure 3). Deletion of either *SFL1* or *HTZ1* caused increased *FLO11* mRNA levels, and accordingly as consequence, adhesiveness of Δ*sfl1*/Δ*sfl1* or Δ*htz1*/Δ*htz1* cells upon glucose limitation was expected (Figure 3). The loss of neither the remodeler encoding gene *ISW2* (Δ*isw2*/Δ*isw2*) nor the repressor encoding gene *SSN6* (Δ*ssn6*/Δ*ssn6*) does change the adhesive growth at glucose limitation, suggesting that they are both dispensable for silencing.

In contrast, the *RSC1* gene is required for adhesiveness after five days of growth on YPD (Figure 3). Even after extended periods of 7 to 10 days of growth on solid medium, resulting in a more pronounced local glucose limitation, Δ*rsc1*/Δ*rsc1* cells do not adhere to the agar surface (not shown). A similar phenotype was observed for Δ*gcn5*/Δ*gcn5* cells lacking the histone H3 acetyl transferase activity encoded by *GCN5*. Those cells also do not grow adhesively but remain in the non-adhesive growth mode (Figure 3).

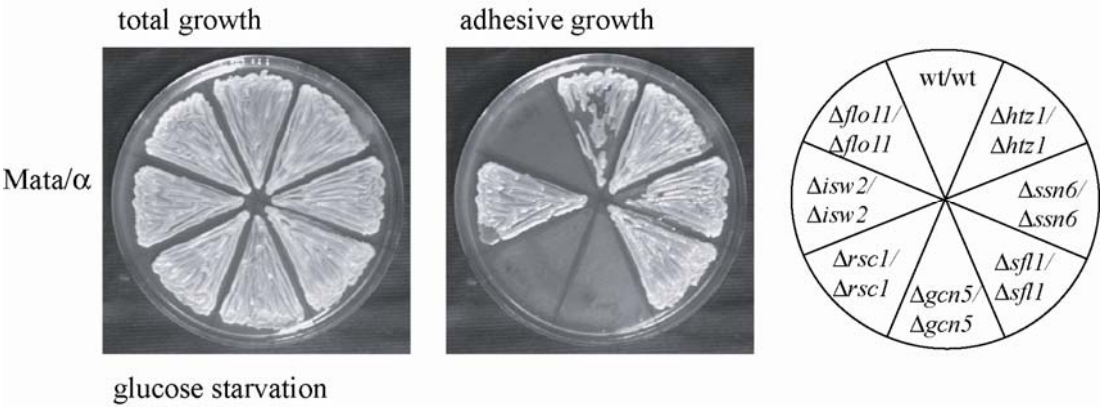

Figure 3. Adhesion of diploid *S. cerevisiae* depends on Rsc1p and Gcn5p. Yeast strains were patched on YPD medium, incubated and photographed after five days of growth (total growth), washed under a stream of water and re-photographed to document remaining cells on agar surface (adhesive growth). Adhesive growth of Δ*isw2*/Δ*isw2* (RH3189), Δ*rsc1*/Δ*rsc1* (RH3186), Δ*gcn5*/Δ*gcn5* (RH3192), Δ*ssn6*/Δ*ssn6* (RH3219), Δ*sfl1*/Δ*sfl1* (RH3222), and Δ*htz1*/Δ*htz1* (RH3216) mutant strains was compared to diploid homozygous yeast strains wt/wt (RH2656) and Δ*flo11*/Δ*flo11* (RH2661) as controls.

Besides adhesion, the formation of pseudohyphae upon nitrogen limitation of Σ1278b cells is strictly *FLO11*-dependent. We therefore tested the potential for pseudohyphal growth at our deletion strains. During differentiating from the yeast form to the pseudohyphal growth mode, *S. cerevisiae* colonies show frayed borders due to an elongated cell morphology and polarized cell growth manifesting as branching chains spreading away from the colony centre (Lo and Dranginis, 1998).

Gene deletion of *SFL1*, *SSN6* or *ISW2* did not affect normal pseudohyphal development, which is in accordance with diploid adhesion as described before. However, in accordance to the adhesion data, Δ*rsc1*/Δ*rsc1* or Δ*gcn5*/Δ*gcn5* cells are unable to form filament-like structures, and therefore, are no longer able to grow as pseudohyphae (Figure 4). Δ*htz1*/Δ*htz1* cells hardly grow on low nitrogen medium, and therefore, could not be analysed for pseudohyphal development.

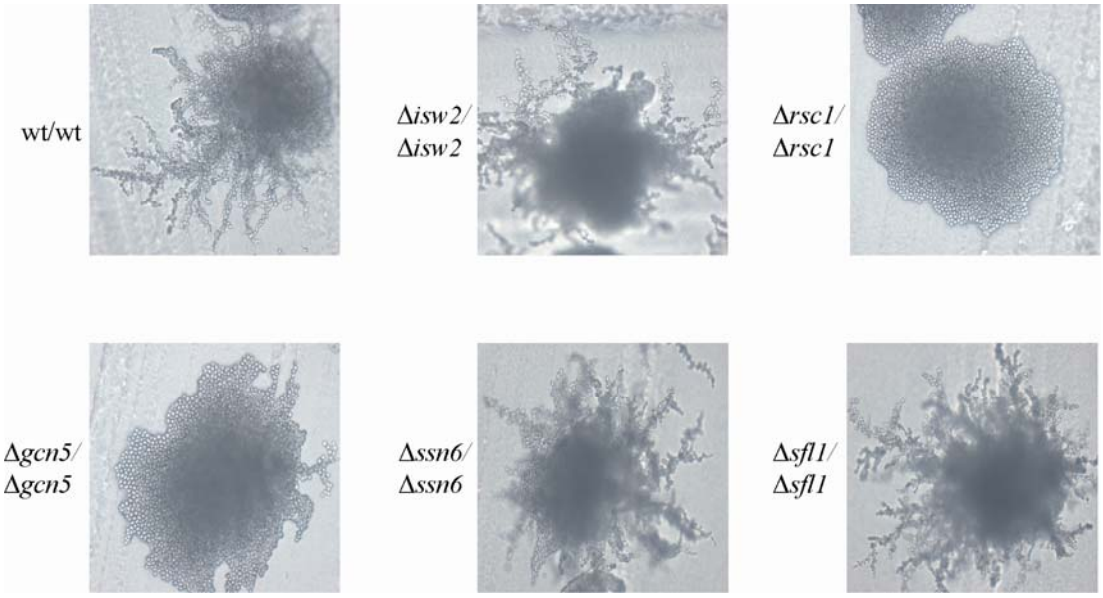

Figure 4. Colony phenotypes of diploid yeast mutant strains impaired in genes for chromatin modifying complexes. Diploid homozygous *S. cerevisiae* strains were streaked out on nitrogen starvation (SLAD) plates to induce pseudohyphal growth and were incubated for five days at 30°C. Colony phenotypes were microscopically examined. wt/wt (RH2656) as control, Δ*isw2*/Δ*isw2* (RH3189), Δ*rsc1*/Δ*rsc1* (RH3186), Δ*gcn5*/Δ*gcn5* (RH3192), Δ*ssn6*/Δ*ssn6* (RH3219) and Δ*sfl1*/Δ*sfl1* (RH3222), respectively, were tested. The Δ*htz1*/Δ*htz1* (RH3216) strain was not included into the assay due to its strong growth defect.

In summary, a functional Rsc1p-containing RSC chromatin remodeling complex and a functional Gcn5p-containing histone acetyltransferase complex are required for both diploid adhesive growth and pseudohyphal development.

The histone variant H2A variant H2A.Z encoding gene *HTZ1* is required to maintain partial repression of *FLO11* expression in haploid yeast cells

The data presented so far originated from diploid yeast cells. As ploidity affects *FLO11* expression (Galitski *et al.*, 1999), we further determined *FLO11* mRNA levels for our set of gene deletions for haploid cells of both mating types. The *FLO11* expression in haploid cells cultivated in rich medium is only partially repressed if compared to diploid cells (Figure 2A). However, this partial repression is not sufficient to cause adhesion after a short growth period of only one day (Figure 5B).
The *SFL1* repressor gene and the histone variant H2A.Z encoding gene *HTZ1*, which are important for *FLO11* repression and silencing in diploid cells are also required to maintain partial *FLO11* repression in haploid cells of both mating types. The *FLO11* mRNA levels of Δ*sfl1* cells rise by a factor of three in both mating types if compared to wildtype *FLO11* expression. Δ*htz1* cells also show increased *FLO11* expression, especially pronounced in a-cells (Figure 5A). Consistently, the increased *FLO11* expression of haploid Δ*htz1*, as well as haploid Δ*sfl1* is reflected in a hyper-adhesiveness phenotype. Both Δ*htz* and Δ*sfl1* cells adhere to the agar surface even after a short growth period, whereas wildtype cells are non-adhesive (Figure 5B). The deletion of either *SSN6* or *GCN5* also resulted in significant mating type-dependent differences in *FLO11* expression. These gene deletions have no significant impact on *FLO11* mRNA levels in α-cells, but a strong impact on a-cells, where *FLO11* transcript levels rise significantly (Figure 5A). Surprisingly, the increased *FLO11* mRNA levels in a-cells of Δ*gcn5* or Δ*ssn6* mutants do not result in hyper-adhesive growth (Figure 5B), suggesting additional control mechanisms of *FLO11* expression.
In summary, the repressor Sfl1p and the histone variant Htz1p are not only required to repress *FLO11* gene expression in diploid cells, but are also necessary for keeping partial *FLO11* repression in haploid cells. Furthermore, there seem to be mating-type specific differences in the control of *FLO11* expression in haploid cells which manifest in the increased *FLO11* transcript levels of Δ*htz1*, Δ*gcn5* and Δ*ssn6* in a- or α-cells.

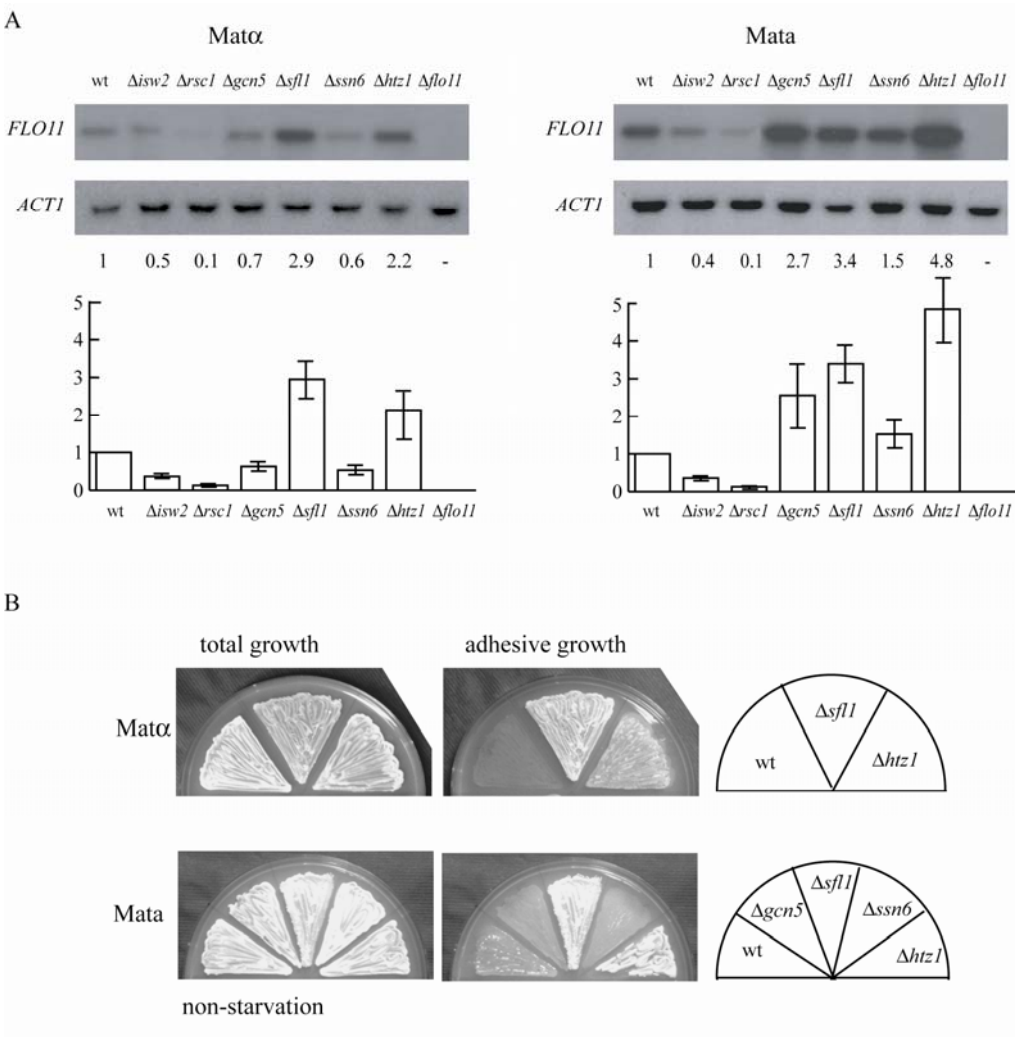

Figure 5. *FLO11* mRNA levels and adhesive growth of various haploid mutant yeast strains. A. Total RNAs were prepared from haploid yeast strains with a- or α-mating type grown in complete YPD medium to logarithmic phase. As control the respective wt strains (RH2817 and RH2818) and a *flo11* deletion strain (RH2662) were used. In addition, Δ*isw2* (RH3187 and RH3188), Δ*rsc1* (RH3184 and RH3185), Δ*gcn5* (RH3190 and RH3191), Δ*ssn6* (RH3217 and RH3218), Δ*sfl1* (RH3220 and RH3221), and Δ*htz1* (RH3214 and RH3215) transcripts were compared. For detection of *FLO11* and *ACT1* transcript levels, 20 µg of total RNAs from each sample were subjected to Northern hybridization analysis. ^{32}P-labeled *FLO11* and *ACT1* (control) riboprobes were used for hybridization. Signals were quantified using a phosphorimaging scanner (Fuji Photofilm & Co. Ltd., Japan). Numbers given indicate relative expression levels of *FLO11* when compared to *ACT1* transcript as internal standard with a value for the wt control strain set as 1. **B.** Haploid *S. cerevisiae* strains with increased *FLO11* mRNA level were tested for accelerated adhesion. Mata cells of Δ*ssn6* (RH3218) and Δ*gcn5* (RH3191), and both mating types of Δ*sfl1* (RH3220 and RH3221), Δ*htz1* (RH3214 and RH3215), and the controls wt (RH2817 and RH2818) and Δ*flo11* (RH2662) were plated on YPD and

incubated for one day at 30°C when the wildtype as control has not yet developed an adhesive phenotype. Non-adhesive yeast cells were washed off the agar surface by rinsing water.

Flo11p-mediated adhesion of haploid yeast cells depends on Rsc1p and Gcn5p in both mating types and on Ssn6p and Isw2p in α-cells

Adhesion of diploid yeast cells and pseudohyphal development rely on derepressed *FLO11* transcription, and depend on Rsc1p and Gcn5p (Figure 3 and 4). Since adhesion is even more pronounced in haploid than in diploid cells, we investigated the impact of our set of gene deletions for haploid adhesive growth upon glucose limitation after three to five days of growth on rich solid medium (YPD).

Δ*rsc1* cells cultivated in rich medium show significantly reduced *FLO11* mRNA levels if compared to wildtype cells (Figure 5A). Upon glucose limitation, substantial *FLO11* derepression in Δ*rsc1* cells is impaired since adhesion is completely abolished for both mating types (Figure 6A). *GCN5* deletion also prevents adhesion in cells of both mating types. However, high *FLO11* transcript levels in Δ*gcn5* α-cells, and approximately wildtype levels in Δ*gcn5* a-cells in the absence of glucose limitation (Figure 5A) suggest an additional mating type specific role of *GCN5* in regulation of *FLO11* expression.

Deletion of *ISW2*, encoding a component of the Isw2p-Itc1p chromatin remodeling complex, gives rise to reduced *FLO11* transcript levels in both mating types (Figure 5A). However, cells of the a-mating type with the auxotrophic marker *leu2* are still able to grow adhesively, whereas α-mating type cells with the auxotrophic marker *trp1* have lost this ability (Figure 6A). This raises the question, whether *FLO11* is differentially transcribed in a- and α-cells. Besides the mating type, also the two different auxotrophic markers *leu2* and *trp1* used in this study for a- or α-cells, might affect *FLO11* transcription. Basal *FLO11* mRNA levels for both markers are by a factor of 1.5 higher in a-cells than in α-cells (Figure 6B). However, in the presence of the *leu2* mutation, the *FLO11* mRNA level is increased by a similar factor (Figure 6B). This suggests that *FLO11* expression depends on the auxotrophic markers.

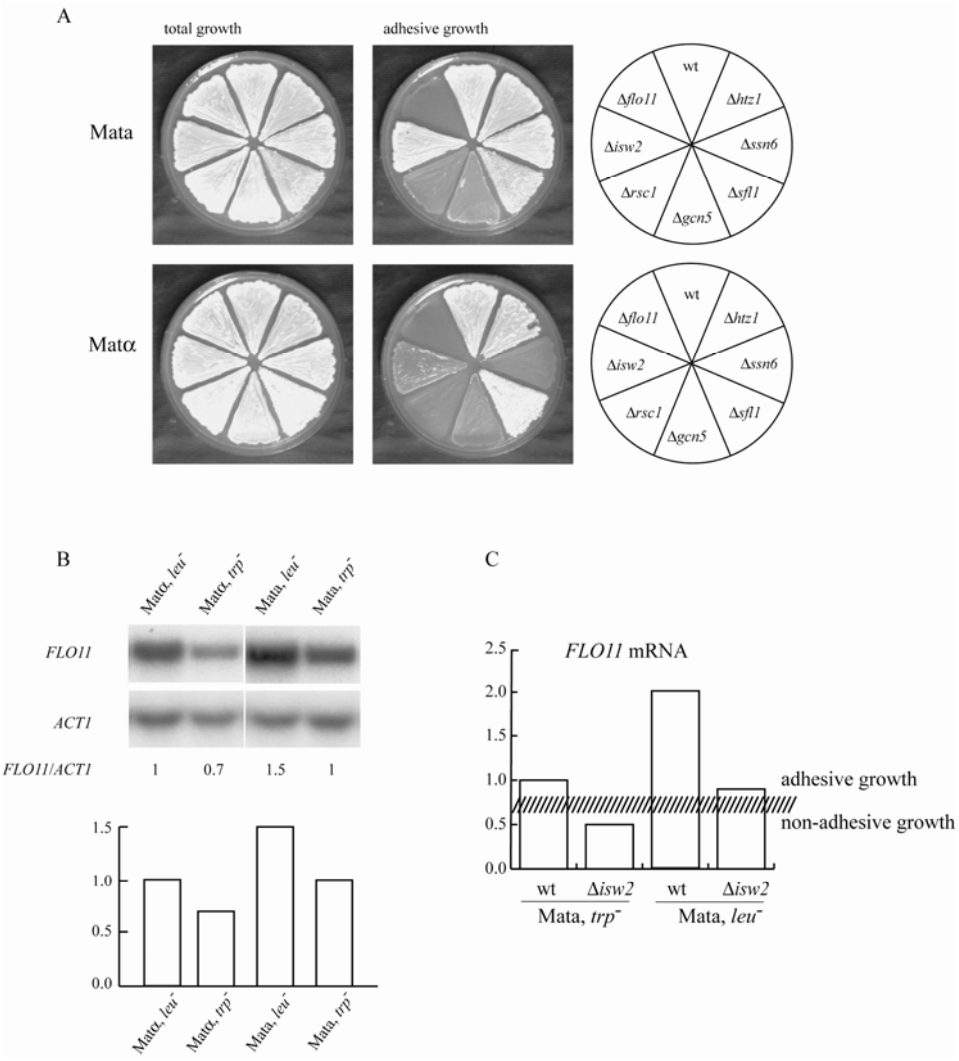

Figure 6. Mutations in *RSC1*, *GCN5*, *ISW2*, or *SSN6* impair haploid adhesive growth of *S. cerevisiae* cells. A. Yeast strains to be tested were patched on YPD medium, incubated and photographed after five days of growth (total growth), washed under a stream of water and re-photographed to document remaining cells on agar surface (adhesive growth). The following strains of both mating types were cultivated: wt (RH2817 and RH2818) and Δ*flo11* (RH2662) as controls, Δ*isw2* (RH3187 and RH3188), Δ*rsc1* (RH3184 and RH3185), Δ*gcn5* (RH3190 and RH3191), Δ*ssn6* (RH3217 and RH3218), Δ*sfl1* (RH3220 and RH3221), and Δ*htz1* (RH3214 and RH3215). For details of genetic background see Table 2. **B.** Haploid yeast wildtype strains RH2816 (Matα, *leu2*::*hisG*), RH2817 (Matα, *trp1*::*hisG*), RH2818 (Mata, *leu2*::*hisG*), and RH2819 (Mata, *trp1*::*hisG*) were cultivated in YPD medium to logarithmic phase. Total RNAs were prepared from the cells and analysed for their *FLO11* transcription rate. Therefore, 20 µg of total RNA from each sample were subjected to Northern hybridization analyses. Hybridization was performed using a ^{32}P-labeled *FLO11* and *ACT1* riboprobes. Signals were quantified using a phosphorimaging scanner (Fuji Photofilm & Co. Ltd., Japan). Numbers given

indicate relative expression levels of *FLO11* when compared to *ACT1* transcript as internal standard. **C.** Scheme of the *FLO11* transcription rate in wildtype strains (RH2817 and RH2818) and the respective Δ*isw2* cells (RH3187 and RH3188). The shading describes the presumed threshold between adhesive and non-adhesive growth depending on the *FLO11* mRNA level.

Altogether, when comparing Mata *leu2* with Matα *trp1* strains a factor of approximately 2 between both has to be considered. So far, the phenotypes of the chromatin deletions with wildtye strains were compared within a group of strains of the one or the other mating type. In view of the 2-fold higher basal *FLO11* transcription in Mata *leu2* strains, we suppose that the mating type specific differences in adhesion observed for Δ*isw2* cells can be explained by the assumption of a certain *FLO11* mRNA threshold that has to be reached for causing cell-surface adhesion. Normalised by the determined factors (Figure 6B), *FLO11* mRNA levels of Matα *trp1* Δ*isw2* cells, in contrast to those of Mata *leu2* Δ*isw2* cells might remain below a required threshold *FLO11* mRNA value (Figure 6C), and therefore, the effect of *ISW2* deletion is mating-type independent.

For haploid Δ*ssn6* cells, wash tests confirm the mating-type specific differences described before for *FLO11* mRNA levels. a-cells had increased *FLO11* transcript levels, and actually stick to the agar surface upon glucose limitation, whereas α-cells had low *FLO11* mRNA levels leading to a non-adhesive growth phenotype (Figures 5A and 6A). The mating type-specifity of Δ*ssn6* cells was confirmed by tetrad dissection of diploid Δ*ssn6*/Δ*ssn6* cells, and testing the resulting colonies for their growth behaviour. Half of the spores (Mata cells) formed adhesive colonies, the other half formed non-adhesive (Matα cells) (data not shown).

Taken together, both an Rsc1p-containing RSC complex and a functional Gcn5p-containing HAT complex are essential for adhesion in diploid, as well as in haploid *S. cerevisiae* cells. The results for the complex Tup1p-Ssn6p further corroborate that there is additional mating-type specific control mechanisms of *FLO11* expression.

Deletion of *SFL1* is epistatic to *RSC1* deletion in adhesion of *Saccharomyces cerevisiae* cells

Deletions of *RSC1* or *SFL1* have antagonistic effects on adhesive growth in diploids, as well as in haploid cells. Δ*rsc1* cells are defective in adhesive growth under glucose limitation (Figures 3 and 6A), whereas adhesive growth is constitutively present in Δ*sfl1* cells even under non-starvation conditions (Figures 2A and 5B).

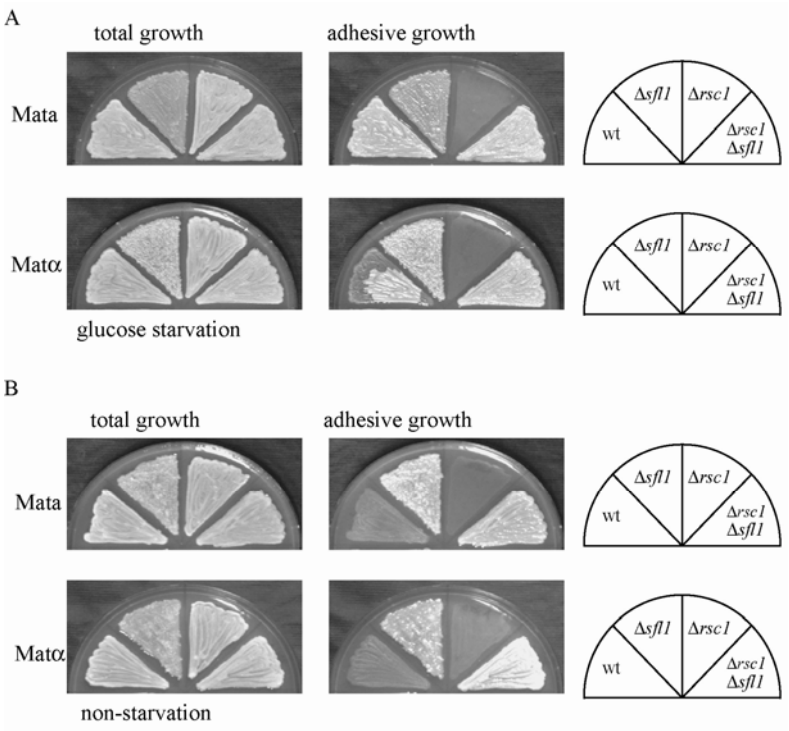

Figure 7. Δ*sfl1* is epistatic to Δ*rsc1* in the adhesive phenotype of *S. cerevisiae* cells. A. Haploid cells of wildtype strains as control (RH2817 and RH2818), Δ*sfl1* (RH3220 and RH3221), and Δ*rsc1* (RH3184 and RH3185) cells, and a Δ*rsc1* Δ*sfl1* double mutant (RH3267 and RH3268) of both mating types, respectively, were patched on YPD medium. Cells were cultivated for a washing assay for five days to induce glucose depletion, and one day to investigate adhesion under non-starvation conditions. **B.** Haploid yeast cells were tested for adhesive growth under non-starvation conditions in a washing assay after only one day of growth on YPD medium. Cells which were cultivated are the same as in A.

We constructed a Δ*rsc1* Δ*sfl1* double mutant by mating haploid single mutants and following sporulation and tetrad dissection. The Δ*rsc1* Δ*sfl1* double mutant was assayed on its ability to grow adhesively to determine the relative strengths of both effects. Deletion of *SFL1* in Δ*rsc1* cells restores the adhesive growth phenotype after 5 days of growth, when glucose is limited in the medium (Figure 7A). Even after growth of only one day on rich medium, Δ*rsc1* Δ*sfl1* cells show adhesive growth, where wildtype cells are non-adhesive (Figure 7B). This suggests a dominant effect of *SFL1* deletion on *FLO11* expression in the absence of *RSC1*.
In summary, the adhesive phenotypes of the Δ*rsc1* Δ*sfl1* double mutant demonstrate that *SFL1* is epistatic to *RSC1*.

RSC1 **supports the Tec1p mediated** ***FLO11***

Tec1p and Flo8p are the final transcription factors at the end of the MAPK cascade and the cAMP/PKA pathway, respectively, and activate *FLO11* transcription (Pan and Heitman, 1999; Rupp *et al.*, 1999). Overexpression of either *TEC1* or *TPK2* is known to stimulate *FLO11* transcription to an extent resulting in the adhesiveness of cells (Köhler *et al.*, 2002; Pan and Heitman, 2002). The *TPK2* encoded catalytic subunit of the cAMP dependent pathway activates Flo8p (Pan and Heitman, 2002). *TEC1* or *TPK2* overexpression plasmids (Table 2) were transformed into Δ*ssn6* cells (Matα), Δ*isw2* cells (Matα), Δ*gcn5* cells (Mata and Matα), or Δ*rsc1* cells (Mata and Matα) deletion strains to analyse whether overexpression complements the adhesion deficient phenotype.
TPK2 overexpression restored adhesiveness of all deletion strains (data not shown), suggesting that the cAMP/PKA pathway can overrule the chromatin factor mediated defects. The MAPK pathway represents by *TEC1* overexpression restored adhesiveness of all deletion strains, except of the Δ*rsc1* mutant strains (Figure 8A). *TEC1* overexpression increases *FLO11* mRNAs in Δ*rsc1* cells (Figure 8B), however, is unable to achieve sufficient levels of *FLO11* mRNA.

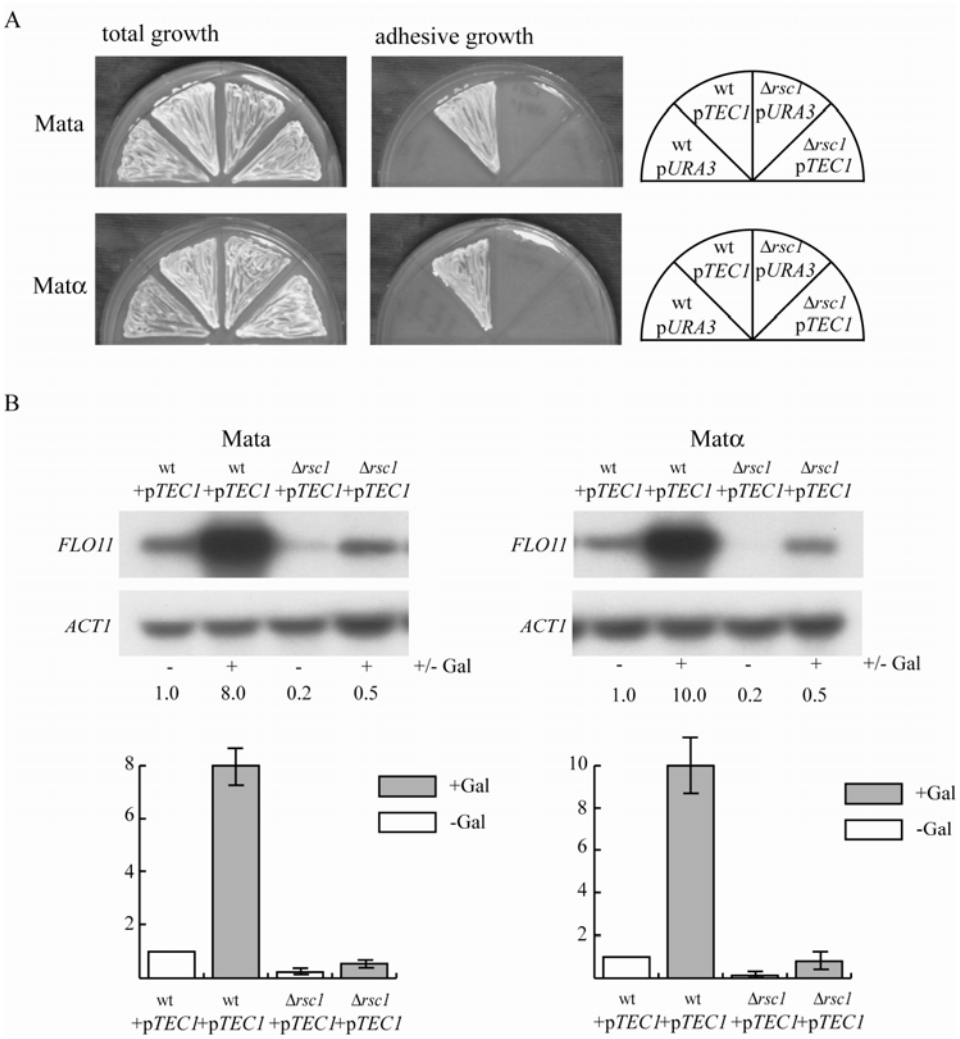

Figure 8. Overexpression of *TEC1* cannot complement the non-adhesive phenotype in Δ*rsc1* yeast mutants. A. Yeast strains to be tested were patched on YNB medium containing 2% galactose and the appropriate supplements. Cells were incubated for five days and photographed before (total growth) and after (adhesive growth) washing the plate with a stream of water to document remaining cells on the agar surface. Following strains were cultivated: RH2817 and RH2818 (wt), as well as RH3184 and RH3185 (Δ*rsc1*) carrying either pRS316 (control) or pME2071 (*GAL-TEC1*). **B.** Haploid yeast strains RH2817 and RH2818 (wt), and RH3184 and RH3185 (Δ*rsc1*) carrying the plasmid pME2071, respectively, were cultivated in YNB medium including 2 % glucose (control) or YNB medium with 2 % galactose to induce overexpression of *TEC1*. Total RNAs were isolated from cells in the logarithmic phase. 20 µg of total RNA from each sample were hybridized with a ^{32}P-labeled *FLO11* and an *ACT1* riboprobe as internal standard. Signals were quantified using a phosphorimaging scanner (Fuji Photofilm & Co. Ltd., Japan). Numbers given indicate relative expression levels of *FLO11* when compared to *ACT1* transcript.

These data suggest a specific requirement for *RSC1* for the MAPK pathway mediated Tec1p activation of *FLO11*.

Discussion

FLO11 transcripts encoding the adhesin required for cell-cell and cell-surface adhesion are hardly detectable in diploid yeast cells during growth on rich medium. Accordingly, the large *FLO11* promoter, which responds to numerous signal transduction pathways depending on external stimuli is silenced by chromatin factors and repressed by DNA-binding proteins in sated cells. The repressor protein Sfl1p has previously been shown to be involved in the transcriptional downregulation of *FLO11* (Robertson and Fink, 1998). In contrast to wildtype yeasts, which show adhesion only upon nutrition limitation signals, diploid Δ*sfl1*/Δ*sfl1* cells and haploid Δ*sfl1* cells of both mating types grow adhesively with or without any nutritional limitations. Our nucleosome mappings of the repressed *FLO11* gene show only one clear MNase-sensitive region within the large promoter. This region coincides with the binding sequence of the repressor Sfl1p and the activator Flo8p (Pan and Heitman, 2002). The rest of the non-expressed *FLO11* promoter is embedded in a protecting chromatin structure. A further MNase-sensitive site presumably corresponds to a promoter region because it is closely located to the divergently oriented neighbouring *MRS1* gene, encoding a splicing factor of mitochondrial RNA (Kreike *et al.*, 1987). Therefore, the chromatin structure of the *FLO11* promoter in diploid cells growing in the non-adhesive yeast mode shares in most parts similarities to silenced chromatin. This silencing is combined with Sfl1p repression. The *FLO11* promoter silencing can be changed to a metastable state of the *FLO11* promoter, where the promoter is either turned “on” or “off” in response to starvation signals which are transduced from the environment to the nucleus. Sfl1p has been reported to directly interact with the co-repressor Ssn6p-Tup1p suggesting a role in the requirement of the co-repressor to respective promoter sites (Conlan and Tzamarias, 2001). It was shown there that deletion of *SSN6* in DBY747 cells of the a-mating type actually derepresses *FLO11* transcription. Although our data confirmed this *FLO11* derepression for Σ1278b cells of a-mating type, Matα and diploid cells of that background did not show any *FLO11* derepression upon *SSN6* deletion. Whereas

diploid Δ*ssn6*/Δ*ssn6* cells are at least able to grow adhesively upon glucose limitation, arguing for intact *FLO11* derepression at this growth condition, haploid Δ*ssn6* cells of the α-mating type remain in a non-adhesive state. Therefore, Tup1p-Ssn6p has opposite effects on *FLO11* expression of the two opposite mating-types.

It has been reported that the ISW2 chromatin remodeling complex collaborates with Ssn6p-Tup1p to organize nucleosome-positioning at the DNA damage-inducible *RNR3* gene (Zhang and Reese, 2004). There, an ordered chromatin structure of promoter and ORF was completely changed in a Δ*isw2* strain, however, without increasing *RNR3* expression. In yeast, the ISW2 complex is rather part of the *FLO11* derepression than of *FLO11* repression and silencing. Its impact on the *FLO11* chromatin structure remains to be investigated.

The histone H2A variant H2A.Z-encoding gene *HTZ1* is genetically required for repression of *FLO11* transcription. *HTZ1* is highly conserved throughout the eukaryotic kingdom. It was suggested to be involved in gene regulation through its involvement in modulating the chromatin structure (Santisteban *et al.*, 2000). Htz1p was shown to positively affect the induction of yeast *GAL* genes, and can affect the interactions of RNA polymerase II-associated factors under specific cell growth conditions (Adam *et al.*, 2001). Accordingly, Larochelle and Gaudreau proposed that Htz1p modulates functional interactions with transcription regulatory components (Larochelle and Gaudreau, 2003), and thus increases their localization to promoter elements affecting the chromatin structure for gene regulation. Microarray data revealed that Htz1p-activated genes cluster near telomers, and further data implicate that Htz1p antagonizes telomeric silencing mediated by *SIR* gene products. The gene product of *HTZ1* presumably enriches in euchromatic regions to form boundary elements preventing the spread of heterochromatin into actively transcribed euchromatin (Meneghini *et al.*, 2003). During early development in mammalian cells, the histone 2A variant H2A.Z is enriched in the pericentric heterochromatin (Rangasamy *et al.*, 2003). Heterochromatin is present around centromeres and telomeres, and among chromosome segregation and genomic stability (Wallrath, 1998) essential for inactivating one of the X-chromosomes in female mammals

(Avner and Heard, 2001). It remains to be elucidated to what extend Htz1p is actually incorporated into the *FLO11* promoter chromatin.

FLO11 expression is silenced in sated diploid cells but only partially repressed in sated haploid cells resulting in a significant basal amount of transcript. This basal haploid *FLO11* expression, which is not sufficient for haploid invasive growth *per se* requires an Rsc1p-containing remodeling RSC complex in a- as well as in α-cells. Rsc1p is also required for *FLO11* induction, and subsequently adhesive growth upon glucose limitation of haploid cells, and for pseudohyphal development upon nitrogen-limitation of diploid cells. Location studies of the SWI-SNF related chromatin remodeler RSC revealed that it preferentially locates in intergenic regions with affinity to Pol III and some Pol II promoters (Ng *et al.*, 2002). Genes of RSC-dependent Pol II promoters are involved in nitrogen and carbohydrate metabolism, and transcriptional regulation. RSC also interacts with the promoters of the histone genes *HTA1* and *HTB1*, and of genes for two subunits of CAC, the chromatin assembly complex. Microarray data of other *RSC* mutant strains also suggest that RSC significantly regulates cell wall genes and that it also down-regulates *FLO11* (Angus-Hill *et al.*, 2001). Data of Bungard *et al.* suggest that RSC also provides signaling information for mid-late sporulation-specific gene expression required for proper spore morphogenesis (Bungard *et al.*, 2004). Huang *et al.* have found that the association of cohesin with centromeres and chromosome arms is differentially regulated by RSC (Huang *et al.*, 2004; Huang and Laurent, 2004). Tandem bromodomains in RSC are supposed to recognize acetylated histone H3 Lys14 in chromatin (Kasten *et al.*, 2004). The mechanism by which RSC stimulates *FLO11* transcription is not cleared in detail. We could show that overexpression of *TEC1*, a further transcription factor of *FLO11* at the end of the MAPK cascade (Rupp *et al.*, 1999), also restored adhesiveness of the natural non-adhesive deletion strains except of the Δ*rsc1* cells. This loss of adhesion and additional low *FLO11* mRNA levels in *TEC1* overexpressing Δ*rsc1* cells might be evidence for a RSC-mediated chromatin remodeling at the *FLO11* promoter required for subsequent Tec1p-promoter binding. However, closer analyses are required to get mechanistical clues for the mode of RSC-action on the *FLO11* promoter.

The determination of *FLO11* mRNA levels of Δ*gcn5* cells showed often deviations among different tested deletion strains. A possible explanation for these *GCN5* specific effects could be the recently described epigenetic regulation of *FLO11* by the histone deacetylase Hda1p, which controls *FLO11* by a heritable *FLO11* state for many generations (Halme *et al.*, 2004). It was discussed there that the observed heterogeneity in *FLO* gene expression, even within a population of cells derived from a single clone, might be due to the activity of Hda1p and its epigenetic consequences. Gcn5p, as potential antagonist of Hda1p, might directly be involved in balancing the appropriate histone acetylation status of the *FLO11* promoter.

The cAMP dependent PKA pathway which is able to activate *FLO11* by the activator Flo8p (Pan and Heitman, 1999) can overrule chromatin defects, and therefore, restore adhesiveness under glucose depletion in all tested non-adhesive deletion strains. This is suggested by the overexpression of the Tpk2p catalytic subunit of the cAMP dependent protein kinase. Therefore, the tested chromatin factors do not seem to be essential for Flo8p mediated activation of *FLO11*.

Taken together, our study revealed that an unusually large promoter as the yeast *FLO11* promoter, which has to be silenced and repressed when diploid cells are sated, but which has to integrate numerous external input signals to be activated when cell-cell adhesion, cell-surface adhesion, invasive growth, biofilm formation or pseudohyphal formation are required, includes multiple levels of regulation, which have to be orchestrated. They include for silencing and repression the chromatin component Htz1p, the chromatin modifier Gcn5p and the repressor component Ssn6p. The activation of Flo11p normally requires the chromatin remodeler Rsc1p and Isw2p in addition to the transcriptional activators.

References

Adam, M., Robert, F., Larochelle, M., and Gaudreau, L. (2001) H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol Cell Biol* **21**: 6270-6279.

Angus-Hill, M.L., Schlichter, A., Roberts, D., Erdjument-Bromage, H., Tempst, P., and Cairns, B.R. (2001) A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin remodeler RSC in gene expression and cell cycle control. *Mol Cell* **7**: 741-751.

Avner, P., and Heard, E. (2001) X-chromosome inactivation: counting, choice and initiation. *Nat Rev Genet* **2**: 59-67.

Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A., and Tan, S. (2002) Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. *J Biol Chem* **277**: 7989-7995.

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**: 115-132.

Braus, G.H., Grundmann, O., Brückner, S., and Mösch, H.U. (2003) Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* **14**: 4272-4284.

Bungard, D., Reed, M., and Winter, E. (2004) *RSC1* and *RSC2* are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*. *Eukaryot Cell* **3**: 910-918.

Cairns, B.R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R.D. (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**: 1249-1260.

Cairns, B.R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R.D., and Winston, F. (1999) Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. *Mol Cell* **4**: 715-723.

Conlan, R.S., and Tzamarias, D. (2001) Sfl1 functions via the co-repressor Ssn6-Tup1 and the cAMP-dependent protein kinase Tpk2. *J Mol Biol* **309**: 1007-1015.

Cross, F.R., and Tinkelenberg, A.H. (1991) A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. *Cell* **65**: 875-883.

Du, J., Nasir, I., Benton, B.K., Kladde, M.P., and Laurent, B.C. (1998) Sth1p, a *Saccharomyces cerevisiae* Snf2p/Swi2p homolog, is an essential ATPase in RSC and differs from Snf/Swi in its interactions with histones and chromatin-associated proteins. *Genetics* **150**: 987-1005.

Fazzio, T.G., Kooperberg, C., Goldmark, J.P., Neal, C., Basom, R., Delrow, J., and Tsukiyama, T. (2001) Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol* **21**: 6450-6460.

Fazzio, T.G., and Tsukiyama, T. (2003) Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. *Mol Cell* **12**: 1333-1340.

Galitski, T., Saldanha, A.J., Styles, C.A., Lander, E.S., and Fink, G.R. (1999) Ploidy regulation of gene expression. *Science* **285**: 251-254.

Gancedo, J.M. (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **25**: 107-123.

Gimeno, C.J., Ljungdahl, P.O., Styles, C.A., and Fink, G.R. (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68**: 1077-1090.

Gray, N.K., and Wickens, M. (1998) Control of translation initiation in animals. *Annu Rev Cell Dev Biol* **14**: 399-458.

Grewal, S.I., and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* **301**: 798-802.

Halme, A., Bumgarner, S., Styles, C., and Fink, G.R. (2004) Genetic and epigenetic regulation of the *FLO* gene family generates cell-surface variation in yeast. *Cell* **116**: 405-415.

Horn, P.J., and Peterson, C.L. (2002) Molecular biology. Chromatin higher order folding--wrapping up transcription. *Science* **297**: 1824-1827.

Huang, J., Hsu, J.M., and Laurent, B.C. (2004) The RSC nucleosome-remodeling complex is required for Cohesin's association with chromosome arms. *Mol Cell* **13**: 739-750.

Huang, J., and Laurent, B.C. (2004) A Role for the RSC chromatin remodeler in regulating cohesion of sister chromatid arms. *Cell Cycle* **3**: 973-975.

Iizuka, M., and Smith, M.M. (2003) Functional consequences of histone modifications. *Curr Opin Genet Dev* **13**: 154-160.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J Bacteriol* **153**: 163-168.

Kasten, M., Szerlong, H., Erdjument-Bromage, H., Tempst, P., Werner, M., and Cairns, B.R. (2004) Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. *EMBO J* **23**: 1348-1359.

Kent, N.A., Karabetsou, N., Politis, P.K., and Mellor, J. (2001) *In vivo* chromatin remodeling by yeast ISWI homologs Isw1p and Isw2p. *Genes Dev* **15**: 619-626.

Kleinschmidt, M., Grundmann, O., Blüthgen, N., Mösch, H.U., and Braus, G.H. (2005) Transcriptional profiling of *Saccharomyces cerevisiae* cells under adhesion-inducing conditions. *Mol Genet Genomics* **273**: 382-393.

Köhler, T., Wesche, S., Taheri, N., Braus, G.H., and Mösch, H.U. (2002) Dual role of the *Saccharomyces cerevisiae* TEA/ATTS family transcription factor Tec1p in regulation of gene expression and cellular development. *Eukaryot Cell* **1**: 673-686.

Kreike, J., Schulze, M., Ahne, F., and Lang, B.F. (1987) A yeast nuclear gene, *MRS1*, involved in mitochondrial RNA splicing: nucleotide sequence and mutational analysis of two overlapping open reading frames on opposite strands. *EMBO J* **6**: 2123-2129.

Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R.A., Pootoolal, J., Tong, A., Canadien, V., Richards, D.P., Wu, X., Emili, A., Hughes, T.R., Buratowski, S., and Greenblatt, J.F. (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell* **12**: 1565-1576.

Lambrechts, M.G., Bauer, F.F., Marmur, J., and Pretorius, I.S. (1996) Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. *Proc Natl Acad Sci U S A* **93**: 8419-8424.

Larochelle, M., and Gaudreau, L. (2003) H2A.Z has a function reminiscent of an activator required for preferential binding to intergenic DNA. *EMBO J* **22**: 4512-4522.

Lee, T.I., Causton, H.C., Holstege, F.C., Shen, W.C., Hannett, N., Jennings, E.G., Winston, F., Green, M.R., and Young, R.A. (2000) Redundant roles for the TFIID and SAGA complexes in global transcription. *Nature* **405**: 701-704.

Lo, W.S., and Dranginis, A.M. (1998) The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol Biol Cell* **9**: 161-171.

Meneghini, M.D., Wu, M., and Madhani, H.D. (2003) Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**: 725-736.

Mösch, H.U., Kübler, E., Krappmann, S., Fink, G.R., and Braus, G.H. (1999) Crosstalk between the Ras2p-controlled mitogen-activated protein kinase and cAMP pathways during invasive growth of *Saccharomyces cerevisiae*. *Mol Biol Cell* **10**: 1325-1335.

Narlikar, G.J., Fan, H.Y., and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**: 475-487.

Neely, K.E., and Workman, J.L. (2002) Histone acetylation and chromatin remodeling: which comes first? *Mol Genet Metab* **76**: 1-5.

Ng, H.H., Robert, F., Young, R.A., and Struhl, K. (2002) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. *Genes Dev* **16**: 806-819.

Pan, X., and Heitman, J. (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Mol Cell Biol* **19**: 4874-4887.

Pan, X., Harashima, T., and Heitman, J. (2000) Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Curr Opin Microbiol* **3**: 567-572.

Pan, X., and Heitman, J. (2002) Protein kinase A operates a molecular switch that governs yeast pseudohyphal differentiation. *Mol Cell Biol* **22**: 3981-3993.

Perez-Martin, J. (1999) Chromatin and transcription in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **23**: 503-523.

Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R., 3rd, and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol Cell Biol* **22**: 8774-8786.

Rangasamy, D., Berven, L., Ridgway, P., and Tremethick, D.J. (2003) Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. *EMBO J* **22**: 1599-1607.

Rangasamy, D., Greaves, I., and Tremethick, D.J. (2004) RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. *Nat Struct Mol Biol* **11**: 650-655.

Roberts, R.L., and Fink, G.R. (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev* **8**: 2974-2985.

Robertson, L.S., and Fink, G.R. (1998) The three yeast A kinases have specific signaling functions in pseudohyphal growth. *Proc Natl Acad Sci U S A* **95**: 13783-13787.

Ruiz, C., Escribano, V., Morgado, E., Molina, M., and Mazon, M.J. (2003) Cell-type-dependent repression of yeast a-specific genes requires Itc1p, a subunit of the Isw2p-Itc1p chromatin remodelling complex. *Microbiology* **149**: 341-351.

Rupp, S., Summers, E., Lo, H.J., Madhani, H., and Fink, G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J* **18**: 1257-1269.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**: 1350-1354.

Santisteban, M.S., Kalashnikova, T., and Smith, M.M. (2000) Histone H2A.Z regulats transcription and is partially redundant with nucleosome remodeling complexes. *Cell* **103**: 411-422.

Sherman, F. (1991) Getting started with yeast. *Methods Enzymol* **194**: 3-21.

Sikorski, R.S., and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19-27.

Smith, R.L., and Johnson, A.D. (2000) Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. *Trends Biochem Sci* **25**: 325-330.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**: 503-517.

Thoma, F., Bergman, L.W., and Simpson, R.T. (1984) Nuclease digestion of circular TRP1ARS1 chromatin reveals positioned nucleosomes separated by nuclease-sensitive regions. *J Mol Biol* **177**: 715-733.

Thoma, F. (1996) Mapping of nucleosome positions. *Methods Enzymol* **274**: 197-214.

Wallrath, L.L. (1998) Unfolding the mysteries of heterochromatin. *Curr Opin Genet Dev* **8**: 147-153.

Wu, P.Y., Ruhlmann, C., Winston, F., and Schultz, P. (2004) Molecular architecture of the *S. cerevisiae* SAGA complex. *Mol Cell* **15**: 199-208.

Yukawa, M., Koyama, H., Miyahara, K., and Tsuchiya, E. (2002) Functional differences between *RSC1* and *RSC2*, components of a for growth essential chromatin-remodeling complex of *Saccharomyces cerevisiae*, during the sporulation process. *FEMS Yeast Res* **2**: 87-91.

Zhang, Z., and Reese, J.C. (2004) Ssn6-Tup1 requires the ISW2 complex to position nucleosomes in *Saccharomyces cerevisiae*. *EMBO J* **23**: 2246-2257.

Chapter 3

Amino acid starvation induced adherence of *Saccharomyces cerevisiae* functions in the absence of transcriptional activation of *FLO11*

Abstract

The *FLO11* gene encodes a cell surface protein which is required for the dimorphic switch from loosely associated cells to multicelluar complexes. In diploid *Saccharomyces cerevisiae* during growth in its non-adherent yeast form, *FLO11* expression is efficiently silenced and repressed. Different stimuli for *FLO11* induction and the subsequent growth phenotypes are known, like glucose, nitrogen or amino acid limitation. Several chromatin factors, including the chromatin remodeler Rsc1p and Isw2p, or the histone acetyl transferase Gcn5p and transcription factors as Tec1p and Flo8p are required for *FLO11* induction. In this study, we analysed a set of chromatin factors on their involvement in amino acid starvation induced adhesion. We find that Δ*rsc1* and Δ*isw2* cells are highly adhesive, although the expression of the *FLO11* gene results in very low mRNA levels. In contrast, the expression of a *FLO11-lacZ* fusion corresponds to a relatively high specific β-galactosidase activity corresponding to the adhesive phenotype. This suggests a post-transcriptional control of *FLO11* expression which might be at the level of translational initiation.

Introduction

A central problem in eukaryotic transcription is how proteins gain access to DNA that is packaged in nucleosomes. Many factors were found to be involved in the process of transcription by remodeling the chromatin structure or assembly the structure between histone proteins and DNA. These factors include histone and histone variants as basic elements of the chromatin structure (Grewal and Moazed, 2003; Horn and Peterson, 2002; Narlikar *et al.*, 2002). Modifications of these histone proteins like acetylation, methylation, phosphorylation or ubiquitination promote loosening the binding to DNA, and therefore, facilitate gene transcription (Iizuka and Smith, 2003). Furthermore, large multisubunit complexes like ISWI or SWI/SNF cause sliding or remodeling of nucleosomes (Narlikar *et al.*, 2002; Perez-Martin, 1999).

The regulation of these factors, which cooperates with the chromatin structure, and other DNA-binding transcription factors correlates with extent of gene transcription. Downstream of transcription, the realization of the coding potential of the genome, including processes from mRNA modification and processing through to protein folding, sorting, transport, and turnover is regulated on a post-transcriptional level. These different modes of regulation are essential for eukaryotic cells and organisms to react on environmental stimuli. A highly regulated gene in the baker's yeast *Saccharomyces cerevisiae* is *FLO11*, coding for a glycosyl-phosphatidylinositol (GPI)-linked cell surface protein. Its expression depends on nutrient supply and allows the cell under certain conditions to switch from loosely associated cells to a multicellular formation and invasive growth (Braus *et al.*, 2003; Gimeno *et al.*, 1992; Lambrechts *et al.*, 1996; Lo and Dranginis, 1998). In diploid yeast cells, *FLO11* is strongly repressed when cells grow as single non-adhesive yeast cells in the absence of nutrient limitations. Beside glucose or nitrogen starvation, also amino acid deficiency overcomes this repression and induces *FLO11* expression, leading to pseudohyphal growth with elongated cells (nitrogen starvation) or adhesive growing cells (glucose or amino acid limitation). Thereby, cells connected with each other by

the *FLO11* adhesin (Gancedo, 2001; Lambrechts *et al.*, 1996; Pan *et al.*, 2000). Also haploid yeast cells respond to glucose or amino acid starvation with a dimorphic switch to the adhesive growth mode, whereas *FLO11* is only partial repressed under non-limiting conditions in contrast to diploids.

Recent studies revealed different new factors of nucleosome architecture, histone modification and nucleosome remodeling, which are required for *FLO11* regulation including both activation and inhibition (see also chapter 2). These studies investigated the impact of these factors on repression and derepression of *FLO11* transcription under non-starvation and glucose-limiting conditions. The histone variant H2A.Z/Htz1p (Santisteban *et al.*, 2000) was identified as an essential component for maintaining the repressive state of the *FLO11* gene in both haploids and diploid *S. cerevisiae* cells. In contrast, activation of *FLO11* transcription, and therefore, adhesive growth or pseudohyphal development requires a functional Rsc1p-containing RSC (remodels the structure of chromatin) complex (Cairns *et al.*, 1996) and a functional Gcn5p-containing histone acetyltransferase complex (Balasubramanian *et al.*, 2002; Pray-Grant *et al.*, 2002; Wu *et al.*, 2004). Whereas not required for diploid filamentous growth, *ISW2*, encoding a component of the Isw2p-Itc1p chromatin remodeling complex (Fazzio *et al.*, 2001), as well as *SSN6*, coding for a component of the co-repressor Ssn6p-Tup1p, show mating-type specific differences in haploid adhesion. Only a-mating type cells need Isw2p or Ssn6p for the dimorphic switch to haploid adhesion (see also chapter 2).

In this study we investigated the impact of chromatin factors on adhesive growth and *FLO11* regulation in both haploid and diploid yeast cells with respect to amino acid starvation by induction with the histidine analogue 3-aminotriazol (3-AT). Furthermore, we determined mutant strains in which only slight *FLO11* expression leads to Flo11p-dependent adhesion under amino acid starvation.

Materials and Methods

Yeast strains and growth conditions

All yeast strains used in this study and their genotypes are listed in Table 1. All strains are derivates of the *S. cerevisiae* Σ1278b background (Gimeno *et al.*, 1992). Deletion mutants were obtained by amplifying the respective deletion cassette, carrying the kan^R marker gene, of the respective BY strain (Brachmann *et al.*, 1998). Transformations were carried out using the lithium-acetate yeast transformation method (Ito *et al.*, 1983). The integrations were confirmed by Southern hybridization analysis (Southern, 1975) or PCR (Saiki *et al.*, 1985). Haploid strains were crossed to produce diploid mutant strains. The Δ*rsc1* Δ*flo11* double mutant strain was constructed by mating RH3184 (Δ*rsc1*::kan^R) and RH3275 (Δ*flo11*::*NAT*) and following tetrad dissection.

Yeast strains were generally cultivated in YPD medium (1 % yeast extract, 2 % bacto peptone, 2 % glucose) at 30°C. For selection of gene disruption 200 µg/ml geneticin G418 was added. For wash tests, cells were grown on synthetic minimal medium (YNB; 0.15 % Yeast Nitrogen Base, 50 mM $(NH_4)_2SO_4$, 200 mM myo-inositol, 20 % glucose, 2 % agar) supplemented with the appropriate amino acids. 3-aminotriazol was added to a final concentration of 10 mM to induce amino acid starvation.

Plasmids

Plasmids used in this study are listed in Table 2. Deletion cassettes were amplified from the respective Euroscarf deletion strain (Brachmann *et al.*, 1998) and inserted via TA-cloning in pBlueskript II SK (Stratagene) using the restriction site *Eco*RV.

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
RH2817	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *HIS* $^+$	Our collection
RH2818	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *HIS* $^+$	Our collection
RH2656	*MAT*a/α, *ura3-52*/*ura3-52*, *trp1*::*hisG*/*TRP1*	Braus *et al*., 2003
RH2662	*MAT*a, *ura3-52*, *Δflo11*::*kan*^{R}	Braus *et al*., 2003
RH3275	*MAT*a, *ura3-52*, *Δflo11*::*NAT*	This study
RH2661	*MAT*a/α, *ura3-52*/*ura3-52*, *trp1*::*hisG*/*TRP1*, *Δflo11*::*kan*^{R}/*Δflo11*::*kan*^{R}	Braus *et al*., 2003
RH3184	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δrsc1*::*kan*^{R}, *HIS* $^+$	This study
RH3185	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δrsc1*::*kan*^{R}, *HIS* $^+$	This study
RH3186	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2* *Δrsc1*::*kan*^{R}/*Δrsc1*::*kan*^{R}, *HIS*$^+$	This study
RH3187	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δisw2*::*kan*^{R} , *HIS* $^+$	This study
RH3188	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δisw2*::*kan*^{R}, *HIS* $^+$	This study
RH3189	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δisw2*::*kan*^{R}/*Δisw2*::*kan*^{R}, *HIS* $^+$	This study
RH3190	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3191	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3192	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δgcn5*::*kan*^{R}/*Δgcn5*::*kan*^{R}, *HIS* $^+$	This study
RH3214	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3215	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3216	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δhtz1*::*kan*^{R}/*Δhtz1*::*kan*^{R}, *HIS* $^+$	This study
RH3217	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δssn6*::*kan*^{R}, *HIS* $^+$	This study
RH3218	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δssn6*::*kan*^{R}, *HIS* $^+$	This study

Table 1. continued

Strain	Genotype	Source
RH3218	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δssn6*::*kan*^{R}, *HIS* $^+$	This study
RH3219	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δssn6*::*kan*^{R}/*Δssn6*::*kan*^{R}, *HIS* $^+$	This study
RH3220	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δsfl1*::*kan*^{R}, *HIS* $^+$	This study
RH3221	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*, *Δsfl1*::*kan*^{R}, *HIS* $^+$	This study
RH3222	*MAT*a/α, *ura3-52*/*ura3-52*, *his3*::*hisG*/*his3*::*hisG*, *trp1*::*hisG*/*TRP1*, *leu2*::*hisG*/*LEU2*, *Δsfl1*::*kan*^{R}/*Δsfl1*::*kan*^{R}, *HIS* $^+$	This study
RH3265	*MAT*α, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δrsc1*:: *kan*^{R}, *Δflo11*:: *NAT*, *HIS* $^+$	This study
RH3266	*MAT*a, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*, *Δrsc1*:: *kan*^{R}, *Δflo11*:: *NAT*, *HIS* $^+$	This study

Table 2. Plasmids used in this study.

Plasmid	Description	Source
pBIISK(+)	Commercial cloning vector	Stratagene, La Jolla, USA
B3782	3 kb *FLO11* promoter fragment in YEp355	(Rupp *et al.*, 1999)
pME2632	pBIISK(+) with integrated deletion cassette of *RSC1* ORF (*kan*^{R})	This study
pME2626	pBIISK(+) with integrated deletion cassette of *ISW2* ORF (*kan*^{R})	This study
pME2628	pBIISK(+) with integrated deletion cassette of *GCN5* ORF (*kan*^{R})	This study
pME2631	pBIISK(+) with integrated deletion cassette of *HTZ1* ORF (*kan*^{R})	This study
pME2648	pBIISK(+) with integrated deletion cassette of *SSN6* ORF (*kan*^{R})	This study
pME2653	pBIISK(+) with integrated deletion cassette of *SFL1* ORF (*kan*^{R})	This study

Genetic crosses, sporulation, and tetrad dissection

Diploid homozygous *S. cerevisiae* strains were obtained by mating of haploid yeast strains that were verified by Southern hybridization experiments. Mating, sporulation and tetrad dissection were performed according to Sherman (Sherman, 1991).

Adhesive growth assays

Adhesive growth of haploid and diploid yeasts were performed as described previously (Roberts and Fink, 1994). Cells were patched on medium in presence and absence of 10 mM 3-AT and incubated for one or five days at 30°C. Plates were photographed to secure total growth and washed under a stream of water. The remaining adhesive cells were documented by photographing the washed plate.

RNA analyis

Total RNAs were isolated from exponentially growing yeast cells according to the method of Cross and Tinkelenberg (Cross and Tinkelenberg, 1991). For Northern hybridization analysis, 20 μg of total RNAs were separated on a 1.4 % agarose gel containing 3 % formaldehyde and transferred onto nylon membranes by capillary blotting. Transcripts of *FLO11* and *ACT1* were detected by using specific ^{32}P-radiolabeled DNA fragments with the Prime-It labelling kit from Stratagene (La Jolla, USA). Signal intensities were visualized and quantified using a BAS-1500 phosphorimaging scanner (Fuji, Tokyo, Japan).

β-Galactosidase Assay

Strains carrying the *FLO11-lacZ* reporter (B3782) were grown in selective media to exponential growth phase. Extracts were prepared and assayed for specific β-galactosidase activity as described previously (Rose and Botstein, 1983) and normalized to the total protein, resulting in the specific enzyme activity (Bradford, 1976).

Results

We analysed the ability of yeast cells to grow adhesively as a result of the environmental signal 'amino acid starvation' for a set of gene deletions, which are known to affect the *FLO11* expression (Chapter 2). The deleted genes are *SFL1*, coding for a known repressor of *FLO11* (Conlan and Tzamarias, 2001), *HTZ1* encoding the histone 2A variant encoding gene (Santisteban *et al.*, 2000), and *SSN6* coding for a component of the co-repressor Ssn6p-Tup1p. Furthermore, *RSC1* and *ISW2* coding for components of the chromatin remodeling complexes RSC and Isw2p-Itc1p, respectively (Cairns *et al.*, 1996; Fazzio *et al.*, 2001), as well as *GCN5*, encoding the histone acetyl transferase activity of various complexes (Balasubramanian *et al.*, 2002; Wu *et al.*, 2004) were deleted.

Amino acid starvation is a strong adhesion signal which restores adhesion of otherwise non-adhesive chromatin deletion strains in yeast

Starvation for amino acids is a strong signal, which causes *FLO11*-dependent adhesion in *S. cerevisiae* (Braus *et al.*, 2003). The set of deletion strains, which have an impact on glucose starvation induced *FLO11* expression, as well as *FLO11* expression in the absence of starvation, were analysed for adhesion and *FLO11* transcription during amino acid starvation (Chapter 2). The aim of this analysis was the question whether different environmental signals result in different effects on *FLO11* expression. Starvation was induced by adding the histidine analogue 3-aminotriazol (3-AT) to the culture medium causing yeast cells to starve for histidine (Hilton *et al.*, 1965).

As controls we used diploid wildtype yeast cells, which grow adhesively after five days of growth on solid rich medium as consequence of local glucose limitations. A further control are deletion strains of *SFL1* or *HTZ1* which result *per se* in adhesive growth after only one day of cultivation, also in absence of 3-AT induced starvation

(Figure 1). In contrast, *RSC1* and *GCN5* are required for adhesiveness after five days of growth in solid rich medium. Cells lacking one of these genes do not even grow adhesively after extended periods of growth on solid medium (see also Chapter 2).

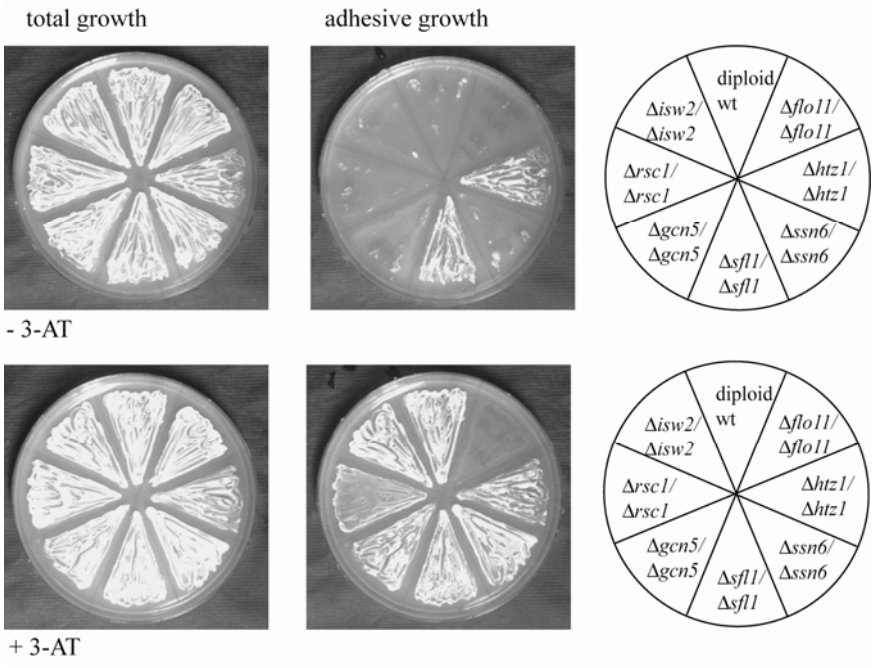

Figure 1. Amino acid starvation suppresses the diploid adhesive growth defects of *S. cerevisiae* deletion mutant strains impaired in *FLO11* expression. Diploid yeast mutant strains were grown on complete YNB medium in the absence or presence of 10 mM 3-AT to induce amino acid starvation. The plates were incubated at 30°C for one day and photographed prior (total growth) and after (adhesive growth) washing off the non-adhesive cells. As control, we used a wt (RH2656) and a *flo11* deletion strain (RH2661). The tested deletion strains were Δ*isw2*/Δ*isw2* (RH3189), Δ*rsc1*/Δ*rsc1* (RH3186), Δ*gcn5*/Δ*gcn5* (RH3192), Δ*ssn6*/Δ*ssn6* (RH3219), Δ*sfl1*/Δ*sfl1* (RH3222), and Δ*htz1*/Δ*htz1* (RH3216).

Strains carrying deletions in *SFL1* encoding a repressor of *FLO11* (Conlan and Tzamarias, 2001), and *HTZ1* coding for the histone variant H2A.Z/Htz1p, (Santisteban *et al.*, 2000), show an adhesive phenotype in solid medium supplemented by 10 mM 3-AT, and therefore, causing amino acid starvation (Figure

1). This corresponds to the phenotype of the same strains under non-starvation or glucose starvation conditions. Cells with deletion of *SSN6* or *ISW2* show also the adhesive phenotype under 3-AT induction. This suggests that the encoding factors Ssn6p and Isw2p are not required for induction of adhesion under amino acid starvation, equally to glucose limitation (Chapter 2). Surprisingly, *RSC1* and *GCN5* which are required for adhesiveness under glucose limitation are not essential for the switch to adhesive growth induced by 3-AT (Figure 1).

Furthermore, we tested the impact of amino acid starvation with 3-AT in haploid strains of both mating types a and α. The constitutive adhesive strains Δ*sfl1* and Δ*htz1* keep on growing adhesive under amino acid starvation (Figure 2). Deletion of *SSN6* leads to mating type specific adhesion under glucose limitation (Chapter 2). In contrast, *SSN6* is not required for adhesion induced with 3-AT leading to amino acid starvation in both mating types (Figure 2). Also Δ*rsc1* and Δ*gcn5* cells respond to amino acid starvation with adhesion.

Altogether, all haploid deletion strains as well as all diploid strains, including the non-adhesive mutants show strong adhesive growth under amino acid starvation (Figure 2).

Summarizing, all diploid deletion strains (Figure 1), as well as the haploid deletion strains (Figure 2) tested here are hyper-adhesive in the presence of 3-AT, in contrast to the Δ*flo11* strain which was used as negative control. Even the strains with deletions in *RSC1* or *GCN5*, respectively, which were strictly non-adhesive at glucose limitation conditions are adhesive under amino acid starvation (Chapter 2). This suggests amino acid starvation as a strong signal, which dominates the effects of the deleted components Ssn6p, Isw2p, Rsc1p, and Gcn5p.

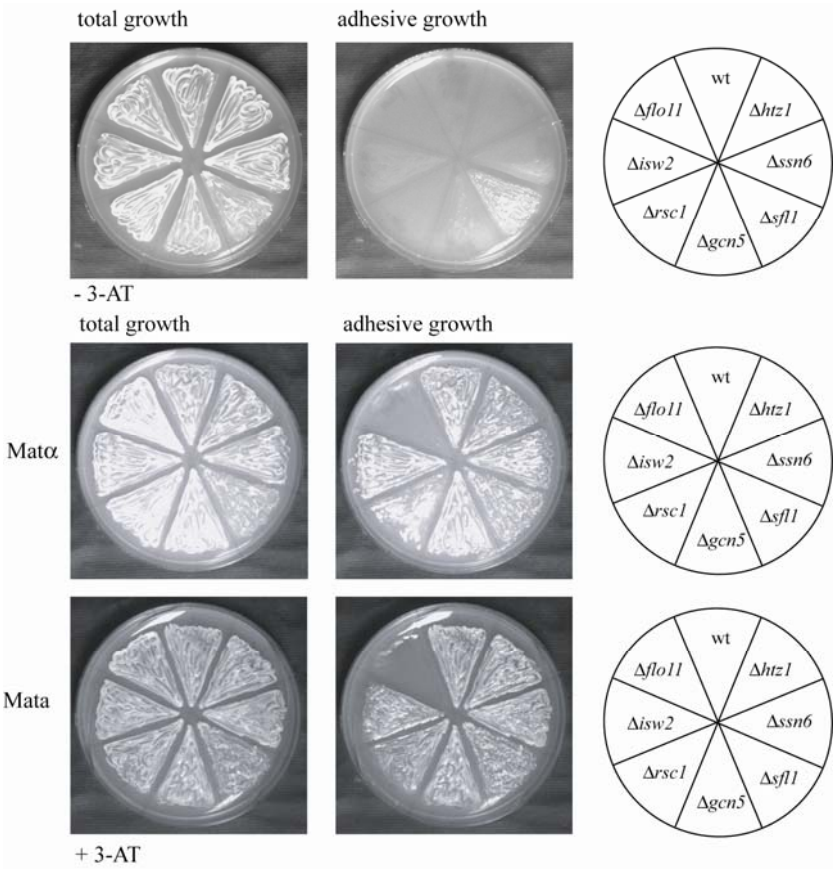

Figure 2. Amino acid starvation suppresses the non-adhesive phenotypes of haploid deletion strains of *S. cerevisiae* impaired in *FLO11* expression. Haploid yeast mutant strains were grown on complete YNB medium in the absence or presence of 10 mM 3-AT to induce amino acid starvation. The plates were incubated at 30°C for one day and photographed before (total growth) and after (adhesive growth) washing off the non-adhesive cells. As control, a wt (RH2817 and RH2818) and a *flo11* deletion strain (RH2662) were used. The tested deletion strains of opposite mating types (Mata and α) were Δ*isw2* (RH3187 and RH3188), Δ*rsc1* (RH3184 and RH3185), Δ*gcn5* (RH3190 and RH3191), Δ*ssn6* (RH3217 and RH3218), Δ*sfl1* (RH3220 and RH3221), and Δ*htz1* (RH3214 and RH3215).

Adhesive growth correlates to very low *FLO11* mRNA levels under amino acid starvation in Δ*rsc1* cells

As described earlier (Braus *et al.*, 2003), *FLO11* mRNA amounts rise approximately five-fold in the presence of 3-AT in wildtype cells. We wanted to test whether adhesion of the set of deletion strains corresponds to their respective *FLO11* mRNA

levels. Figure 3 show Northern experiments which reflect the transcription of *FLO11* in wildtype and all tested deletion strains when incubated in medium with 10 mM 3-AT. As described earlier (Braus *et al.*, 2003), *FLO11* transcription rate increases in presence of 3-AT to a five-fold level. Although a different growth phenotype of the deletion strains under the tested conditions, a similar change of *FLO11* transcription can be observed under amino acid starvation (Figure 3), as it was shown for non-starvation (Chapter 2), which is more intensive in a-cells comparing to α-cells. Deletion of *GCN5*, *SSN6*, *SFL1*, or *HTZ1* leads to a strongly increased *FLO11* transcription in a-cells, whereas in α-cells, the *FLO11* mRNA level remains lower than the induced wildtype level with 3-AT. *FLO11* transcription levels remains very low in Δ*isw2* cells, and especially in Δ*rsc1* cells of both mating types (Figure 3), which is in contrast to the adherence phenotype described above (Figure 1).

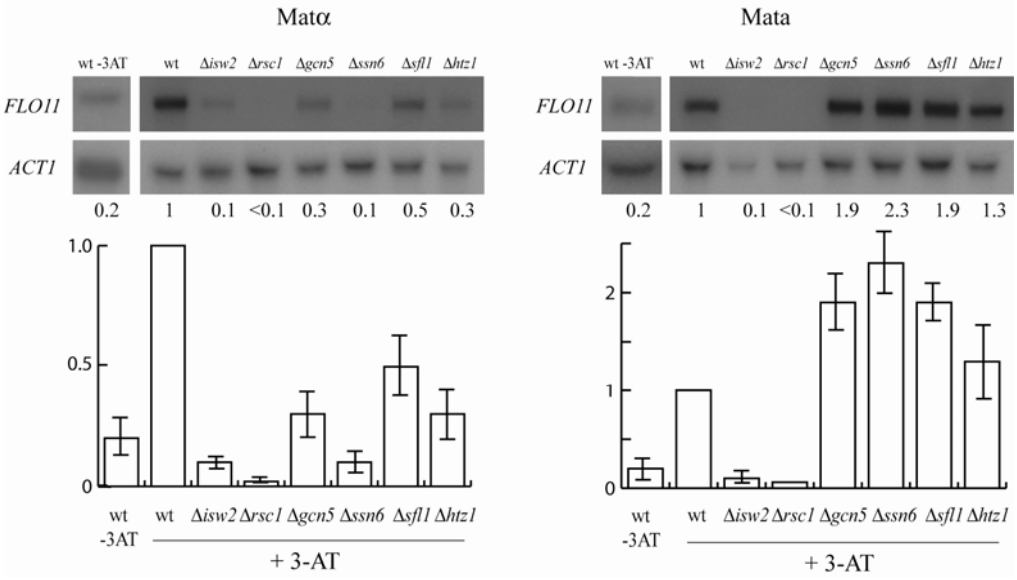

Figure 3. Amino acid induced adhesion does not always correlate to increased *FLO11* transcription rates. Total RNAs were prepared from haploid yeast strains of a- and α-mating type, respectively, grown in complete YNB with 10 mM 3-AT to logarithmic phase. As control the respective wt strains (RH2817 and RH2818) were used. In addition, Δ*isw2* (RH3187 and RH3188), Δ*rsc1* (RH3184 and RH3185), Δ*gcn5* (RH3190 and RH3191), Δ*ssn6* (RH3217 and RH3218), Δ*sfl1* (RH3220 and RH3221), and Δ*htz1* (RH3214 and RH3215) were analysed. For detection of *FLO11* and *ACT1* transcript levels, 20 µg of total RNA from each sample were subjected to

Northern hybridization analyses. Hybridization was performed using a ^{32}P-labeled *FLO11* and *ACT1* riboprobes. Signals were quantified using a phosphorimaging scanner (Fuji Photofilm & Co. Ltd., Japan). Numbers given indicate relative expression levels of *FLO11* when compared to *ACT1* transcript as internal standard with a value for the wt control strain corresponding to 1.

Summarizing, the induction of adhesion with amino acid starvation not solely depends on increased *FLO11* transcription rate. There have to be other mechanisms or factors which are involved in the phenotype switch induced with 3-AT. One possibility is a *FLO11*-independency, where the adhesion is caused by another flocculin that is normally silenced in Σ1278b strains. The alternative way is a continuing *FLO11*-dependency, where translation is sufficiently in spite of a very low mRNA level.

Adhesion of Δ*rsc1* cells during amino acid starvation is *FLO11* dependent and correlate to increased *FLO11-lacZ* reporter activity

We analysed whether the 3-AT induced adhesion in Δ*rsc1* cells without appreciable increased *FLO11* transcription is still *FLO11*-dependent or caused by another flocculin. Therefore, a double mutant was constructed with both deletion of *RSC1* and *FLO11*, and tested for adhesive growth under amino acid starvation. In fact, the washing assay of a Δ*rsc1* Δ*flo11* double mutant strain shows no more adhesion in presence of 3-AT (Figure 4A). Although *FLO11* mRNA remains very low in Δ*rsc1* cells in the presence of 3-AT (Figure 3), adhesiveness of Δ*rsc1* cells at amino acid starvation has still to be *FLO11*-dependent. This suggests that additional post-transcriptional mechanisms might be involved in the control of *FLO11* expression under amino acid starvation conditions.

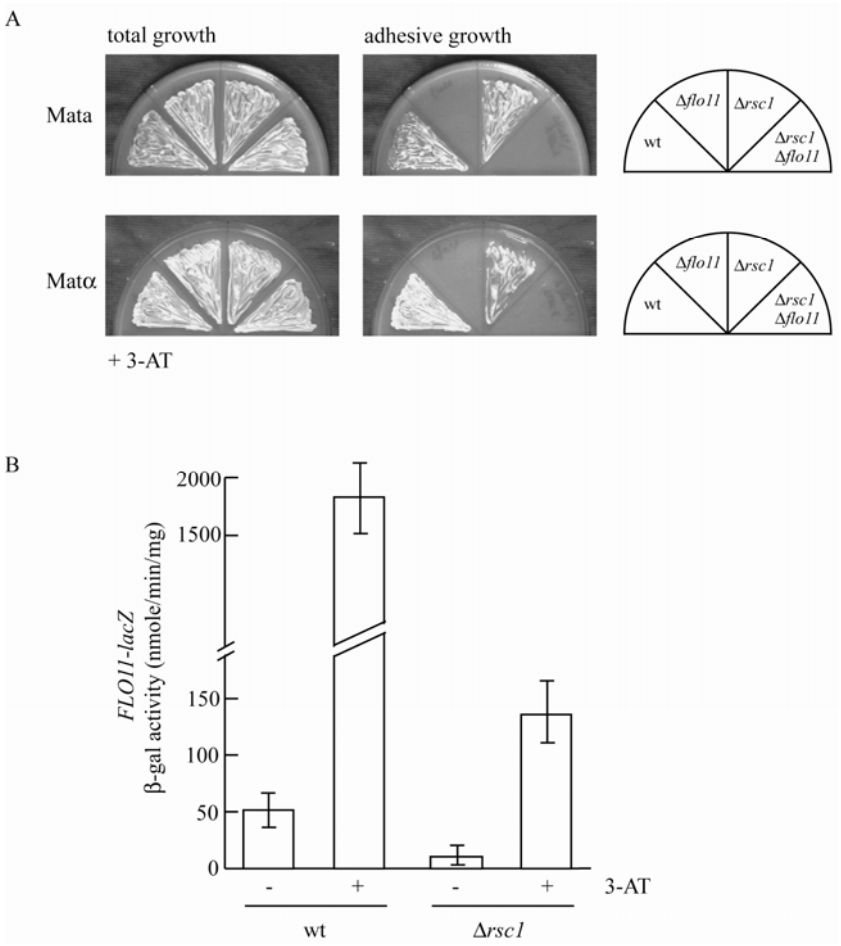

Figure 4. Amino acid induced adhesion in Δ*rsc1* yeast cells correlates to increased *FLO11-lacZ* activity. A. Haploid yeast strains RH2817 and RH2818 (wt), as well as RH2662 (Δ*flo11*) as controls, RH3184 and RH3185 (Δ*rsc1*), and RH3265 and RH3266 (Δ*rsc1* Δ*flo11*) were patched on YNB medium containing 10 mM 3-AT. The plates were incubated at 30°C for one day and photographed before (total growth) and after (adhesive growth) washing off the non-adhesive cells. **B.** Expression of *FLO11-lacZ* reporter gene was measured in haploid yeast strains RH2817 and RH2818 (wt), as well as RH3184 and RH3185 (Δ*rsc1*) carrying plasmid B3782. Specific β-galactosidase activities were measured in absence or presence of 10 mM 3-AT.

The specific β-galactosidase activies of *FLO11-lacZ* reporter genes in the different mutant strains were measured to further support this assumption. Wildtype cells show increased β-galactosidase activity under amino acid starvation comparing to non-starvation conditions, as expected. Only low activity of the *FLO11-lacZ* reporter

gene was measured in Δ*rsc1* cells grown in the presence of sufficient amino acids. High specific β-galactosidase activities of *FLO11-lacZ* reporter genes were observed in Δ*rsc1* cells in the presence of 3-AT, by far exceeding the level of non-induced wildtype cells, although *FLO11* mRNA are very low (Figure 4B). This is in agreement with the *FLO11*-dependent adhesive phenotype, and demonstrates that presumably no other flocculin is involved in adhesion of Δ*rsc1* cells in the presence of 3-AT. This suggests a post-transcriptional mechanism for *FLO11*-derepression upon amino acid starvation which is caused by the 5' untranslated region of the mRNA.

Discussion

Yeast cells respond to certain environmental signals with a dimorphic switch to invasive growing multicellular complexes. Therefore, the expression of the adhesin encoding gene *FLO11* is required (Braus *et al.*, 2003; Gimeno *et al.*, 1992; Lambrechts *et al.*, 1996; Lo and Dranginis, 1998). On rich medium, its transcription is strongly repressed in diploid cells and partially repressed in haploids (Guo *et al.*, 2000). Several pathways mediate different signals to the *FLO11* promoter resulting in overcoming the silent state. These signals are glucose or nitrogen limitation, as well as amino acid starvation or a high amount of certain transcription factors (Braus *et al.*, 2003; Cullen and Sprague, 2000; Gimeno *et al.*, 1992). For regulating this *FLO11* transcription in both activation and silencing or repression also chromatin remodeling factors are involved. Recent studies revealed that a functional Rsc1p-containing RSC complex as well as the histone acetyltransferase Gcn5p, and Ssn6p and Isw2p (only in Matα cells) are required for activation of *FLO11* expression, and therefore, for adhesion under glucose limitation (see chapter 2).

Investigations in this study reveal the effect of 3-aminotriazol induced amino acid starvation, which has a strong impact on *FLO11* dependent adhesion, with respect to the deleted chromatin regulating activities. *FLO11* dependent adhesion upon amino acid starvation seems only partially be due to increased *FLO11* transcription, which is observed for haploid wildtype cells (Braus *et al.*, 2003). Namely Δ*rsc1* cells expressing only very low *FLO11* mRNA levels even at amino acid starvation grow hyper-adhesive at these circumstances. A Δ*rsc1* Δ*flo11* double mutant is no more adhesive under amino acid starvation, suggesting that this effect is *FLO11* dependent. This strongly suggests an efficient derepression mechanism that acts post-transcriptionally, possibly on the level of translation.

An attractive hypothesis is based on the fact that the *FLO11* mRNA contains a putative upstream open reading frame (uORF) that might be involved in a control mechanism of *FLO11* mRNA translation. Mapping the 5' termini of mRNAs of *STA1-3*, which share 97% of the sequence in the promoter region and the first 96 nt

of the ORF with *FLO11* (Lambrechts *et al.*, 1996), suggesting heterogeneity in transcriptional start sites (Lambrechts *et al.*, 1991). One transcriptional start point is positioned 100 nucleotides upstream the ORF, including the uORF positioned -61 to -57 nucleotides (Lambrechts *et al.*, 1991). Due to the high identity of *FLO11* with the *STA* genes, equal transcription start points are presumable but have to be determined.

The presence of initiation codons and uORFs in the 5' upstream region often negatively influences translation of a gene (Kozak, 1991, 1992). Recent studies showed that uORFs affect up to 20% of fungal genes (Galagan *et al.*, 2005). An example where an uORF exerts a regulatory influence on the downstream ORF to determine the selective translation of a protein is the *GCN4* system in *S. cerevisiae* (Hinnebusch, 1994). The influence of an uORF may vary according to the physiological signals. Amino acid starvation generally reduces cellular translation efficiency, but specifically enhances the translation of the *GCN4* mRNA by means of its far uORFs (Grundmann *et al.*, 2001). Cat-1, a mammalian cationic amino acid transporter, is also upregulated upon amino acid starvation conditions, namely on the level of increased transcription, as well as on the level of increased translation of its mRNA (Fernandez *et al.*, 2003). There, it has been shown that the uORF containing Cat-1 mRNA is translationally upregulated by ribosome stalling at the uORF leading to very efficient IRES-mediated translation (Fernandez *et al.*, 2005). Ongoing projects will investigate the putative role of the *FLO11* uORF at different physiological circumstances according to translation efficiency.

Taken together, this study demonstrates that besides a very complex transcriptional *FLO11* regulation also a very efficient post-transcriptional mechanism seems to regulate expression of this adhesin, especially under amino acid starvation conditions.

References

Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A., and Tan, S. (2002) Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. *J Biol Chem* **277**: 7989-7995.

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**: 115-132.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248-254.

Braus, G.H., Grundmann, O., Brückner, S., and Mösch, H.U. (2003) Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* **14**: 4272-4284.

Cairns, B.R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R.D. (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**: 1249-1260.

Conlan, R.S., and Tzamarias, D. (2001) Sfl1 functions via the co-repressor Ssn6-Tup1 and the cAMP-dependent protein kinase Tpk2. *J Mol Biol* **309**: 1007-1015.

Cross, F.R., and Tinkelenberg, A.H. (1991) A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. *Cell* **65**: 875-883.

Cullen, P.J., and Sprague, G.F., Jr. (2000) Glucose depletion causes haploid invasive growth in yeast. *Proc Natl Acad Sci U S A* **97**: 13619-13624.

Fazzio, T.G., Kooperberg, C., Goldmark, J.P., Neal, C., Basom, R., Delrow, J., and Tsukiyama, T. (2001) Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol* **21**: 6450-6460.

Fernandez, J., Lopez, A.B., Wang, C., Mishra, R., Zhou, L., Yaman, I., Snider, M.D., and Hatzoglou, M. (2003) Transcriptional control of the arginine/lysine transporter, cat-1, by physiological stress. *J Biol Chem* **278**: 50000-50009.

Fernandez, J., Yaman, I., Huang, C., Liu, H., Lopez, A.B., Komar, A.A., Caprara, M.G., Merrick, W.C., Snider, M.D., Kaufman, R.J., Lamers, W.H., and Hatzoglou, M. (2005) Ribosome stalling regulates IRES-mediated translation in eukaryotes, a parallel to prokaryotic attenuation. *Mol Cell* **17**: 405-416.

Galagan, J.E., Calvo, S.E., Cuomo, C., Ma, L.J., Wortman, J., Batzoglou, S., Lee, S.I., Brudno, M., Batürkmen, M., Spevak, S.S., Clutterbuck, J., Kapitonov, V., Jurka, J., Scazzocchio, C., Farman, M., Butler, J., Purcell, S., Harris, S., Braus, G.H., Draht, O., Busch, S., D'Enfert, C., Bouchier, C., Goldman, G.H., Griffith-Jones, S., Vienken, K., Pain, A., Selker, E.U., Archer, D., Penalva, M.A., Oakley, B.R., Momany, M., Sano, M., Tanaka, T., Kumagai, T., Machida, M., Nierman, W.C., Denning, D.W., Caddick, M., Hynes, M., Paoletti, M., Fischer, R., Miller, B., Dyer, P., Sachs, M.S., Osmani, S.A., and Birren, B. (2005) Sequencing and comparative analysis of *Aspergillus nidulans*. *Nature*. Under Revision.

Gancedo, J.M. (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **25**: 107-123.

Gimeno, C.J., Ljungdahl, P.O., Styles, C.A., and Fink, G.R. (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68**: 1077-1090.

Grewal, S.I., and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. *Science* **301**: 798-802.

Grundmann, O., Mösch, H.U., and Braus, G.H. (2001) Repression of *GCN4* mRNA translation by nitrogen starvation in *Saccharomyces cerevisiae*. *J Biol Chem* **276**: 25661-25671.

Guo, B., Styles, C.A., Feng, Q., and Fink, G.R. (2000) A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc Natl Acad Sci U S A* **97**: 12158-12163.

Hilton, J.L., Kearney, P.C., and Ames, B.N. (1965) Mode of action of the herbicide, 3-amino-1,2,4-triazole(amitrole): inhibition of an enzyme of histidine biosynthesis. *Arch Biochem Biophys* **112**: 544-547.

Hinnebusch, A.G. (1994) Translational control of *GCN4*: an in vivo barometer of initiation-factor activity. *Trends Biochem Sci* **19**: 409-414.

Horn, P.J., and Peterson, C.L. (2002) Molecular biology. Chromatin higher order folding--wrapping up transcription. *Science* **297**: 1824-1827.

Iizuka, M., and Smith, M.M. (2003) Functional consequences of histone modifications. *Curr Opin Genet Dev* **13**: 154-160.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J Bacteriol* **153**: 163-168.

Kozak, M. (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. *J Biol Chem* **266**: 19867-19870.

Kozak, M. (1992) A consideration of alternative models for the initiation of translation in eukaryotes. *Crit Rev Biochem Mol Biol* **27**: 385-402.

Lambrechts, M.G., Pretorius, I.S., Sollitti, P., and Marmur, J. (1991) Primary structure and regulation of a glucoamylase-encoding gene (STA2) in Saccharomyces diastaticus. *Gene* **100**: 95-103.

Lambrechts, M.G., Bauer, F.F., Marmur, J., and Pretorius, I.S. (1996) Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. *Proc Natl Acad Sci U S A* **93**: 8419-8424.

Lo, W.S., and Dranginis, A.M. (1998) The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol Biol Cell* **9**: 161-171.

Narlikar, G.J., Fan, H.Y., and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**: 475-487.

Pan, X., Harashima, T., and Heitman, J. (2000) Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Curr Opin Microbiol* **3**: 567-572.

Perez-Martin, J. (1999) Chromatin and transcription in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **23**: 503-523.

Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R., 3rd, and Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol Cell Biol* **22**: 8774-8786.

Roberts, R.L., and Fink, G.R. (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev* **8**: 2974-2985.

Rose, M., and Botstein, D. (1983) Construction and use of gene fusions to *lacZ* (beta-galactosidase) that are expressed in yeast. *Methods Enzymol* **101**: 167-180.

Rupp, S., Summers, E., Lo, H.J., Madhani, H., and Fink, G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J* **18**: 1257-1269.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**: 1350-1354.

Santisteban, M.S., Kalashnikova, T., and Smith, M.M. (2000) Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. *Cell* **103**: 411-422.

Sherman, F. (1991) Getting started with yeast. *Methods Enzymol* **194**: 3-21.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**: 503-517.

Wu, P.Y., Ruhlmann, C., Winston, F., and Schultz, P. (2004) Molecular architecture of the *S. cerevisiae* SAGA complex. *Mol Cell* **15**: 199-208.

Chapter 4

Mutual regulation of the isogenic ribosomal partners Rps26Ap and Rps26Bp on translational level in *Saccharomyces cerevisiae*

Abstract

The small 40S ribosomal subunit of yeast is composed of 32 proteins. The *RPS26* isogenes of *Saccharomyces cerevisiae*, which are located on chromosome V and VII, respectively, encode for two proteins of the small subunit of the ribosome. Both *RPS26* genes share a high degree of sequence identities and the deduced proteins differ only by two amino acids. Expression studies of the *FLO11* gene, encoding an adhesion required for filamentous growth revealed that only *RPS26A* but not *RPS26B* is required for translation of *FLO11* mRNA. Strains with deletions in *RPL16A* and *RPL16B* of the large ribosomal subunit show that the adhesive phenotype is not affected, and therefore, the non-adhesive phenotype in Δ*rps26A* cells is specific to the respective protein. Construction of hybrid genes encoding tagged versions of the isogenic proteins constricts their function in differentiation processes in yeast. Investigation with these reporter constructs suggest that translation of the *RPS26A* and *RPS26B* mRNAs depends on each other in a reciprocal manner. The presence of Rps26Ap stimulates Rps26Bp formation, whereas Rps26Bp has the opposite effect.

Introduction

Ribosomes are complex macromolecular machineries present in all living cells that translate messenger RNAs (mRNAs) into the respective proteins. In the eukaryotic model system *Saccharomyces cerevisiae*, 80S ribosomes consists of a large 60S subunit which is built by the 25S, 5.8S, 5S rRNAs and 46 proteins, and an additional smaller 40S subunit consisting of the 18S rRNA and 32 proteins (Green and Noller, 1997; Spahn *et al.*, 2001).

Rps26p of *S. cerevisiae* represents one of the proteins of the small 40S subunit where the exact positioning within or at the ribosome is yet unknown (Gomez-Lorenzo *et al.*, 2000; Planta and Mager, 1998). Rps26p is encoded by two isogenes, a situation which is similar for many ribosomal protein encoding genes (Mager *et al.*, 1997; Planta and Mager, 1998). The open reading frames of the yeast isogenes *RPS26A* on chromosome VII and *RPS26B* on chromosome V share 97% of sequence identities and code for two almost identical proteins of 119 amino acids. The deduced Glu^{106} and Asp^{113} residues of Rps26Ap are substituted for Asp^{106} and Ala^{113} in Rps26Bp. Cytoplasmic ribosomes seem to share Rps26p with mitochondrial ribosomes although no homologous gene for a corresponding prokaryotic ribosomal protein is known (Koc *et al.*, 2000).

In yeast, at least one of the *RPS26* isogenes is essential for growth, a Δ*rps26A*/Δ*rps26B* double mutation exhibits a synthetic lethal phenotype (Strittmatter, 2003). Beside the protein sequence, the gene expression level of the two isogenic partners is different, due to the necessity of *RPS26A* for normal growth of the yeast cell. *RPS26A* alone delivers a major part of the *RPS26* transcript level, whereas *RPS26B* alone supplies only a minor part of the *RPS26* transcript (Strittmatter, 2003). To fulfill various steps of yeast differentiation including Flo11p-dependent adherence or filamentous growth, a certain threshold protein level of Rps26p seems to be required which can only be provided by the presence of *RPS26A* (Strittmatter, 2003). An increased sensitivity towards paromomycin in a *RPS26B*

over-expressing yeast strain hints to an additional yet unknown function of Rps26Bp (Strittmatter, 2003).

Here, we provide evidence that the amount of Rps26p within a *S. cerevisiae* cell is a bottleneck for the translation of the *FLO11* transcript, which is required for filamentous growth. Furthermore, we analysed whether the two isogenic partners Rps26Ap and Rps26Bp affect the gene expression of each other. Therefore, we determined the expression rate with promoter-*lacZ* fusions, as well as the protein levels in different strain backgrounds with tagged versions of the respective protein and found that the two genes influence the gene expression of each other in a reciprocal way.

Materials and Methods

Yeast strains, plasmids and growth conditions

Yeast strains used in this study are listed in Table 1 and are derivates of the *S. cerevisiae* Σ1278b. Used plasmids are listed in Table 2. Strains were cultivated in YPD medium or in synthetic complete (SC) medium as described earlier (Miozzari *et al.*, 1978). Transformations were carried out using the lithium-acetate method (Ito *et al.*, 1983). All deletions and integrations were confirmed using the Southern hybridization analysis (Southern, 1975).

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
RH2584	*MAT*a, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	Our collection
RH2588	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*	Our collection
RH2590	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	Our collection
RH2592	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *trp1*::*hisG*	Our collection
RH2594	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	Our collection
RH2613	*MAT*a, *ura3-52*::750 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2614	*MAT*a, *ura3-52*::1400 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2615	*MAT*a, *ura3-52*::1080 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2621	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::750 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2622	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::1400 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2623	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::1080 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2629	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::750 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2630	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::1400 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2631	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::1080 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study

Table 1. continued

Strain	Genotype	Source
RH2662	*MAT*a, *ura3-52*, *Δflo11*::*kan*^{R}	Braus *et al.*, 2003
RH2878	*MAT*a, *ura3-52*::1050 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2879	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::1050 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2880	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::1050 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2881	*MAT*a, *ura3-52*::420 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2882	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::420 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2883	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::420 bp p*RPS26A*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2884	*MAT*a, *ura3-52*::540 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2885	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::540 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH2886	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::540 bp p*RPS26B*::*lacZ*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3176	*MATα, ura3-52*::*myc*^{3}*-RPS26A*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3177	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::*myc*^{3}*-RPS26A*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3178	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::*myc*^{3}*-RPS26A*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3179	*MATα, ura3-52*::*myc*^{3}*-RPS26B*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3180	*MAT*a, Δ*rps26A*::*kan*^{R}, *ura3-52*::*myc*^{3}*-RPS26B*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3181	*MAT*a, Δ*rps26B*::*kan*^{R}, *ura3-52*::*myc*^{3}*-RPS26B*::*URA3*, *his3*::*hisG*, *trp1*::*hisG*	This study
RH3277	*MAT*a, Δ*rps17B*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	This study
RH3279	*MAT*a, Δ*rpl16A*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	This study
RH3281	*MAT*a, Δ*rpl16B*::*kan*^{R}, *ura3-52*, *his3*::*hisG*, *leu2*::*hisG*	This study

Table 2. Plasmids used in this study.

Plasmid	Description	Source
pRS316	*URA3*, *CEN*, *bla*, *ori*	Sikorski and Hieter, 1989
YIplac211	*URA3*, 3.8 kb integrative vector	Myers *et al.*, 1986
YIp355	*URA3*, 7.1 kb *lacZ* shuttle vector	Myers *et al.*, 1986
B3782	3 kb *FLO11*::*lacZ* fusion in YEp355	Rupp *et al.*, 1999
pME2144	YIp355 with 750 bp p*RPS26A*-*lacZ* fusion as *Eco*RI fragment	This study
pME2146	YIp355 with 1400 bp p*RPS26B*-*lacZ* fusion as *BamH*I fragment	This study
pME2148	YIp355 with 1080 bp p*RPS26B*-*lacZ* fusion as *Eco*RI fragment	This study
pME2405	YIp355 with 1050 bp p*RPS26A*-*lacZ* fusion as *Eco*RI fragment	This study
pME2406	YIp355 with 420 bp p*RPS26A*-*lacZ* fusion as *Eco*RI fragment	This study
pME2407	YIp355 with 540 bp p*RPS26B*-*lacZ* fusion as *Eco*RI fragment	This study
pME2707	YIplac211 with myc^3-*RPS26A* fusion as *Xba*I/*Eco*RI fragment	This study
pME2709	YIplac211 with myc^3-*RPS26B* fusion as *Xba*I/*Kpn*I fragment	This study

Yeast adherence assay

Haploid adhesive growth assays of *S. cerevisiae* strains were performed as described previously (Roberts and Fink, 1994). Cells were patched on the respective medium and incubated for five days at 30°C. Total growth was secured by photographing the plate. Plates were washed under a stream of water and the remaining cells were photographed again.

Northern hybridization analysis

Total RNAs were prepared from exponentially growing cells as described earlier (Cross and Tinkelenberg, 1991). About 20 µg of total RNAs were compared to 10 µg of total RNAs of yeast wildtype and Δ*rps26B* strains, respectively, to compensate the Δ*rps26A* growth defect. *FLO11* and *ACT1* transcripts were detected by hybridization with gene specific ^{32}P-radiolabeled DNA probes. Hybridization signals were quantified using a Fuji Bas-1500 imager (Fuji Photofilm & Co. Ltd., Japan).

Construction of *RPS26A* and *RPS26B* promoter-*lacZ* fusions

Three promoter fragments of *RPS26A* varying in length (420 bp, 750 bp, and 1050 bp) were fused in frame to the *lacZ* reporter gene using *Eco*RI restriction site in YIp355 (Myers *et al.*, 1986). Resulting plasmids are pME2405, pME2144, and pME2406 (Table 2). Accordingly, parts of the *RPS26B* promoter of three different lengths were fused to the *lacZ* reporter gene. 540 bp and 1080 bp were fused as *Eco*RI fragments, whereas the 400 bp fragment was fused as *Bam*HI fragment in YIp355 (Myers *et al.*, 1986), resulting in plasmids pME2146, pME2148, and pME2407 (Table 2). Plasmids were linearised by *Stu*I digestion and were chromosomally integrated into the *ura3-52* locus of the yeast genome of RH2584, RH2588, and RH2592. Single integration was confirmed by Southern analysis (Southern, 1975).

β-Galactosidase assay

S. cerevisiae cells were cultured in liquid media to exponential growth phase. Extracts were prepared and assayed for specific β-galactosidase activity as described previously (Rose and Botstein, 1983). Specific β-galactosidase activity was

normalized to the total protein level in each extract to result in specific enzyme activities (Bradford, 1976).

Construction of triple myc epitope tagged versions of yeast Rps26Ap and Rps26Bp

Plasmids pME2707 and pME2709 expressing a triple *myc* epitope-tagged (myc^3-) version of either Rps26Ap or Rps26Bp under the control of its own promoter were obtained by insertion of a 120 bp *Bam*HI fragment carrying the triple myc^3-epitope after the third amino acid, resulting in fusion proteins which are carrying tags which are located in close proximity to the N-terminus. The fusion construct of *RPS26A* was cloned with 750 bp of the promoter region and 550 bp of the termination region as *Xba*I/*Eco*RI-fragment in the integrative vector Ylplac211. The fusion construct of *RPS26B* was cloned with 1.4 kb of the promoter region and 500 bp of the termination region as *Xba*I/*Kpn*I-fragment in the same vector. The plasmids were linearized with *Stu*I for integration in the *ura3-52* locus of strains RH2584, RH2588, and RH2592, respectively. Functional integration restored a functional *URA3* copy.

Protein analysis

Preparation of whole yeast cell extracts was performed as described previously (Surana *et al.*, 1993). Routinely, 10 µg of crude protein extract were separated on sodium dodecyl-sulfate gels. Proteins were transferred to nitrocellulose membrane. After incubation of the membranes with polyclonal mouse anti-myc and a peroxidase-coupled goat anti-mouse secondary antibody, the proteins were visualized using ECL technology (Amersham, Freiburg, Germany).

Results

The non-adhesive growth phenotype of *Saccharomyces cerevisiae* is specific for loss of Rps26p and do not reflect the loss of functional ribosome

Recent studies showed that Δ*rps26A* yeast mutant strains are impaired in developmental programs for haploid adhesive and diploid pseudohyphal growth. These mutant cells produce a non-adhesive phenotype in haploid cells under glucose starvation and missing pseudohyphal formation under nitrogen limittion, whereas the absence of *RPS26B* exhibits no phenotype different from the wildtype (Strittmatter, 2003).

We asked whether the non-adhesive phenotype is specific to the loss of Rps26Ap or whether this is a general translational effect resulting from a constricted working ribosome. Therefore, we deleted further ribosomal protein encoding genes and analysed them on their effect on the adhesive phenotype. Genes which were deleted are *RPL16A* and *RPL16B* coding for proteins of the large subunit of the yeast ribosome, as well as *RPS17B* encoding a representative component of the small ribosomal subunit (Abovich *et al.*, 1985; Lecompte *et al.*, 2002; Planta and Mager, 1998). As described recently (Strittmatter, 2003), the deletion of *RPS26B* has no effect on the adhesive phenotype, in contrast to the deletion of *RPS26A*, which resulted in a non-adhesive strain (Figure 1). Washing assays with Δ*rps17B*, Δ*rpl16A* and Δ*rpl16B* cells showed that these genes are not essential for adhesive growth when cells were incubated on rich medium for at least five days to induce local glucose depletion.

This suggests a specific effect on adhesive growth when *RPS26A* is deleted and not a general effect resulting from an imperfectly working ribosome which could decrease the whole translational process.

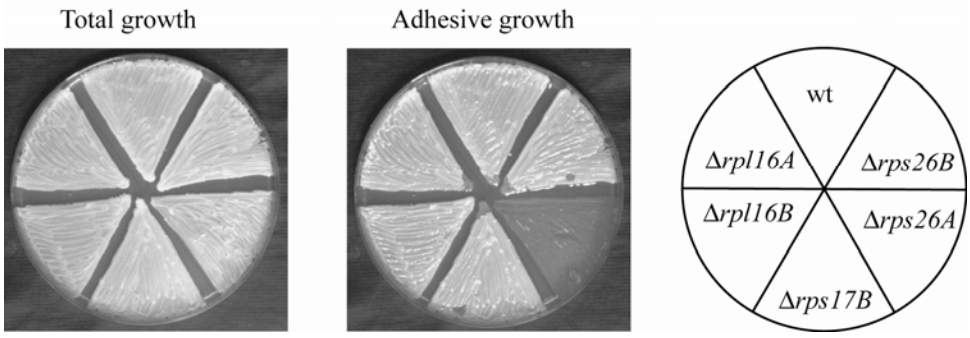

Figure 1. Adhesive growth of different yeast mutant strains with deletions in ribosomal components. Yeast strains to be tested were patched on YPD medium, incubated and photographed after five days of growth (total growth), washed under a stream of water and re-photographed to document remaining cells on the agar surface (adhesive growth). The used strains were wt (RH2584), Δ*rps26A* (RH2590), and Δ*rps26B* (RH2594) as controls, Δ*rps17B* (RH3277), Δ*rpl16A* (RH3279), and Δ*rpl16B* (RH3281). For details of genetic background see Table 1.

FLO11-lacZ activity is abolished and *FLO11* mRNA levels are reduced in haploid Δ*rps26A* yeast mutant strains

Flo11p is one of the key factors in the control of haploid adhesive and diploid pseudohyphal growth (Lambrechts *et al.*, 1996). Recent studies showed that Δ*rps26A* yeast mutant strains are impaired in these developmental programs by producing a non-adhesive phenotype in haploid cells and missing pseudohyphal formation, whereas the absence of *RPS26B* exhibits no phenotype different from the wildtype (Strittmatter, 2003). We analysed the *FLO11* transcription level as well as the p*FLO11-lacZ* activity of the wildtype and in various Δ*rps26* mutant strains to find out whether these phenotypes depend on *FLO11* expression (Figure 2A+B). All strains were carrying an intact *FLO11* and in addition the p*FLO11-lacZ* reporter construct (B3782).

Comparing the *FLO11* transcript levels in different strain backgrounds, we found that transcription rate of *FLO11* in yeast wildtype cells is similar to that of Δ*rps26B* yeast mutant strains (Figure 2A). The amounts of β-galactosidase-activities derived from the p*FLO11-lacZ* reporter are different comparing wildtype and Δ*rps26B* mutant strains. Wildtype cells exhibit specific β-galactosidase-activities of about 145

nmol/min/mg, whereas Δ*rps26B* mutant strains yield only 30% reduced β-galactosidase-activities of about 90 nmol/min/mg (Figure 2B). *FLO11* gene transcription as well as mRNA expression are also affected by the absence of *RPS26A*, but less strong as on translational level. The *FLO11* mRNA level is reduced in Δ*rps26A* mutant cells when compared to the wildtype (Figure 2A). In agreement, the specific β-galactosidase-activities in this strain background are strongly reduced and undetectable (Figure 2B).

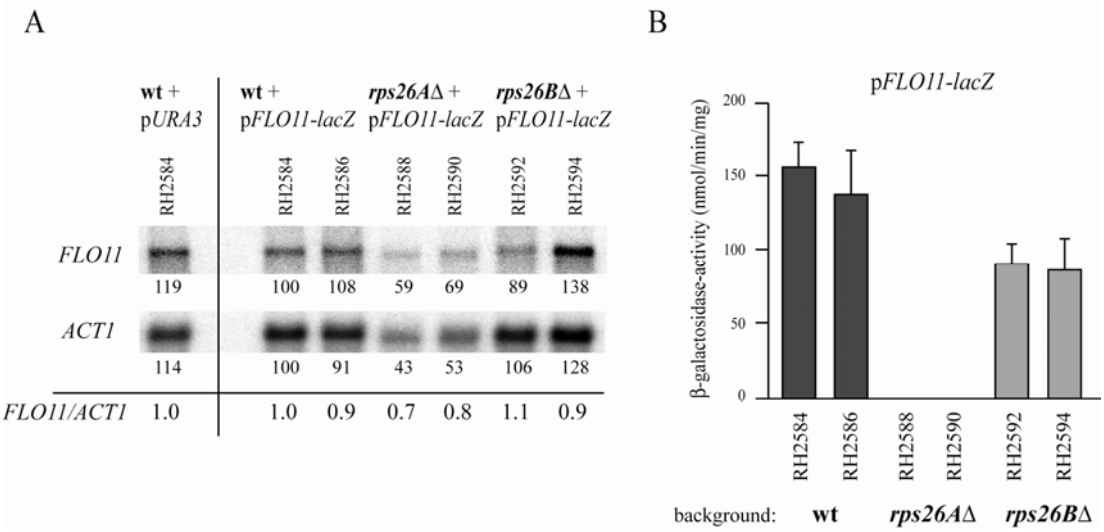

Figure 2. *FLO11* transcription and *FLO11-lacZ* activity in Δ*rps26A* and Δ*rps26B* yeast strains. A. Total RNAs were prepared from haploid yeast strains RH2584 and RH2586 (wt), RH2588 (Δ*rps26A::Kan*^{R}) and RH2590 (Δ*rps26A::Kan*^{R}), and RH2592 (Δ*rps26B::Kan*^{R}) and RH2594 (Δ*rps26B::Kan*^{R}), respectively, all carrying the *FLO11* reporter construct B3782 (+p*FLO11-lacZ*). As plasmid control pRS316 (+p*URA3*) was taken. 10 µg of total RNAs were hybridized to ^{32}P-labeled riboprobes of *FLO11* and *ACT1*. Signals were quantified using a Phosphor-Imager. Numbers depicted give average counts of three independent measurements, with standard deviations not exciting 15%. Below, the average ratios of the *FLO11/ACT1* transcript levels of three measurements are given. **B.** The averages of the specific β-galactosidase activities of the six strains mentioned above are depicted, with standard deviations indicated.

These data suggest that a Δ*rps26A* yeast mutant strain is unable to express Flo11p from its mRNA. This might be due to the low levels of the ribosomal Rps26p protein of the small ribosomal subunit, which is only provided by the *RPS26B* gene, which is only weakly expressed. This suggests a specific function of Rps26p for *FLO11* translation. The Δ*rps26B* mutant strain still exhibits sufficiently high amounts of Rps26p to allow at least a reduced expression of the *FLO11*. Therefore, the amount of Rps26p seems to be a bottleneck for the dimorphic switch in the baker's yeast.

An intact *RPS26A* decreases *RPS26A-lacZ* activity in yeast, while *RPS26B* increases *RPS26B-lacZ* activity

The *S. cerevisiae* genes *RPS26A* and *RPS26B* are differently expressed. There might be additional differences between *RPS26A* and *RPS26B*, which were determined by the sensitivity towards the drug paromomycin, which increases the translational error rate by decreasing the dissociation of t-RNAs bound to the A-site of the ribosome (Carter *et al.*, 2000). A *RPS26B* over-expressing yeast strain showed an increased sensitivity towards paromomycin, which was not observed in a *RPS26A* over-expressing yeast strain (Strittmatter, 2003). Therefore, we aimed to impact of the presence or absence of *RPS26A* and *RPS26B* on the respective gene expression to analyse whether the two genes affect each other.

Different *RPS26A* and *RPS26B* promoter-*lacZ* fusions were constructed to analyse the extent of the regulating promoter region, respectively. A typical promoter of *S. cerevisiae* is about 300 bp to 500 bp in length (Mewes *et al.*, 1997), whereas large promoters as *e. g.* the *FLO11* promoter span about 3 kb in length (Rupp *et al.*, 1999). Therefore, different lengths of the respective upstream region were tested, including the small open reading frames (uORFs) upstream of the actual open reading frames (ORFs), which might affect gene expression. The three different promoter fragments of *RPS26A* were fused in-frame to the *lacZ*-gene, respectively, as well as the three promoter-fragments of the *RPS26B* gene locus (Figure 3). All constructs were chromosomally integrated into the *URA3* locus of a wildtype strain (RH2584) with presence of both, *RPS26A* and *RPS26B*, and in absence of one of the *RPS26* genes

(RH2588 and RH2592), respectively. We determined the translational activities in all 18 resulting derivative yeast strains.

We found that the 420 bp promoter fusion of the *RPS26A* gene resulted in very low specific β-galactosidase activities in all tested strains (Figure 3A). This suggests that the 420 bp fragment does not include the entire promoter resulting in only partial *RPS26A* gene expression. p*RPS26A-lacZ* fusions with 750 bp or 1050 bp of the upstream region related to the start codon resulted in almost identical specific β-galactosidase activity values in the respective strain backgrounds. This indicates that 750 bp of the 5' region comprises the complete promoter region of *RPS26A* with at least one upstream activation site (UAS) between positions -420 bp and -750 bp relative to the start codon of *RPS26A*. Furthermore, this demonstrates that the upstream uORF (*YGL188C*) presumably does not affect the correct expression of the *RPS26A* gene. Comparing the specific β-galactosidase activities in the different backgrounds, we found that *RPS26A-lacZ* expression is almost identical in wildtype strain background and in Δ*rps26B* mutant strains. In cells with deleted *RPS26A* and p*RPS26A-lacZ* fusion, the specific β-galactosidase activity values were raised to about 1.5 fold when compared to wildtype or Δ*rps26B* mutant strains (Figure 3A). These results suggest an inhibition of *RPS26A-lacZ* expression by Rps26Ap.

The specific β-galactosidase activity values of strains with 540 bp p*RPS26B-lacZ* fusion resulted only in weak p*RPS26B-lacZ* expression in all tested strains (Figure 3B). This suggests that the complete promoter comprises more than 540 bp for full *RPS26B* expression. In all strain backgrounds the specific β-galactosidase activity increases when *lacZ* is expressed by 1080 bp of the upstream region of *RPS26B*. A further increase of p*RPS26B-lacZ* expression resulting in maximal β-galactosidase activity causes a 1400 bp comprising promoter. This suggests a promoter length of at least 1080 bp and at most 1400 bp including several upstream activation sites (UAS) to be involved in *RPS26B* expression. At least one is located between positions -540 bp and -1080 bp relative to the start codon of *RPS26B*, as well as between positions -1080 bp and -1400 bp. Comparing the p*RPS26B-lacZ* expression in the different strain backgrounds, similar specific β-galactosidase activities can be observed between wildtype yeast and Δ*rps26A* cells (Figure 3B). In contrast, the values of

specific β-galactosidase activity in Δ*rps26B* cells are reduced to less than half. These results suggest that Rps26Bp is required for full expression of *RPS26B-lacZ*.

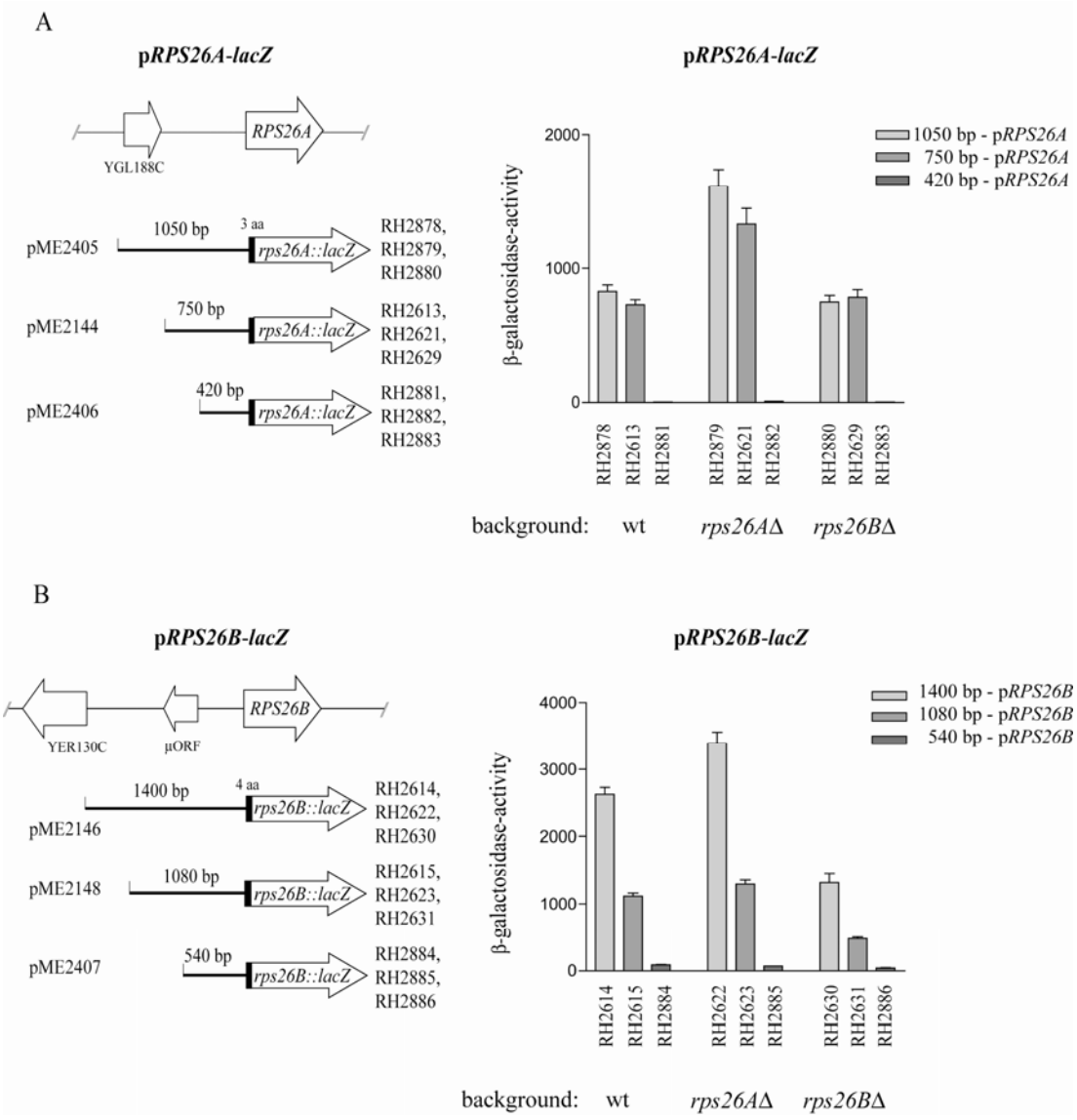

Figure 3. Expression of *RPS26A-lacZ* and *RPS26B-lacZ* fusions in *S. cerevisiae* integrated at the *URA3* gene locus. A. Three plasmids were constructed carrying a 420 bp (pME2406), 750 bp (pME2144), or a 1050 bp (pME2405) promoter fragment of the *RPS26A* gene locus fused in frame to the *lacZ* reporter gene. These constructs were linearised by *Stu*I digestion and were chromosomally integrated into strains RH2584 (wildtype), RH2588 (Δ*rps26A::Kan*^{R}) and RH2592 (Δ*rps26B::Kan*^{R}), respectively. Resulting yeast strains carrying the p*RPS26A-lacZ* reporter constructs at the *URA3* locus are given with their respective strain numbers. On the right side,

the average values of three independent measurements of the β-galactosidase activities are depicted with indicated standard deviations. **B.** Three plasmids were constructed carrying a 540 bp (pME2407), 1080 bp (pME2148), or a 1400 bp (pME2146) promoter fragment of the *RPS26B* gene locus fused in frame to the *lacZ* reporter gene. These constructs were linearised by *Stu*I digestion and were chromosomally integrated into strains RH2584 (wildtype), RH2588 (Δ*rps26A::Kan*^{R}) and RH2592 (Δ*rps26B::Kan*^{R}), respectively. Resulting strains carrying the p*RPS26A-lacZ* reporter constructs at the *URA3* locus are given as described in Table 1. On the right side, the average values of three independent measurements of the specific β-galactosidase activities are depicted with indicated standard deviations.

Summarizing the results obtained from the promoter analysis, we found that regulator elements for gene transcription of *RPS26B* are located inbetween at least 1400 bp upstream the ORF, whereas the promoter region of *RPS26A* span only about 740 bp. Furthermore, the impact on the corresponding reporters suggests that Rps26Ap impairs its own translation initiation, whereas Rps26Bp seems to stimulate its own translation initiation.

High amounts of Rps26Ap increase expression of *RPS26B*, while high amounts of Rps26Bp decrease the expression of *RPS26A*

The *lacZ*-data (Figure 3) with respect to the transcriptional levels of the *RPS26* genes (Strittmatter, 2003), suggest that both *RPS26* gene products seem to affect their own expression on a post-transcriptional level. The effect is significantly different with Rps26Bp and Rps26Ap enhancing and decreasing their own expression, respectively. We determined the protein levels of both *RPS26* gene products to further corroborate these results. Therefore, a set of in frame N-terminal tagged triple *myc-RPS26A* and triple *myc-RPS26B* alleles was constructed (pME2707, pME2709). *Myc*^{3}-tagged alleles were integrated into the yeast chromosome at the *URA3* locus of strain RH2584 (wildtype), RH2588 (Δ*rps26A::Kan*^{R}) and RH2592 (Δ*rps26B::Kan*^{R}), respectively. All resulting strains were tested for protein levels in a Western analysis. In a pre-test the function of N-terminal modified Rps26Ap and Rps26Bp was tested in an adhesive growth assay. An Δ*rps26A* yeast mutant strains is unable to perform

haploid adhesive growth, whereas the absence of *RPS26B* exhibits no influence on adherence. In our wash test, neither the C-terminal modifications (data not shown) nor the N-terminal modified triple *myc-RPS26A* (*myc*^{3}*-RPS26A*) is able to completely restore adhesive growth in an Δ*rps26A* yeast mutant strain (Figure 4A). Therefore, the modified Rps26Ap (*myc*^{3}*-RPS26A*) seems to interfere with its own function. Although the modified *RPS26* proteins are only partially functional, they were used as further reporter to verify or falsify *lacZ*-data (Figure 3). With the determination of the protein levels we revealed interesting insights into the regulation of the *RPS26* gene expression.

Expression of a *myc*^{3}-tagged *RPS26A* allele in the genetic wildtype background in presence of both proteins the native Rps26Ap and the native Rps26Bp, is strongly inhibited and not detectable (Figure 4B, lane 1). However, the *myc*^{3}-Rps26B protein shows a different effect and results in significant expression (Figure 4B, lane 4). The expression level of *myc*^{3}-Rps26Ap was also low in the presence of native Rps26Bp within the Δ*rps26A* background (Figure 4B, lane 2). Therefore, native Rps26Bp without Rps26Ap seems to be unable to result in appropriate *myc*^{3}*-RPS26A* gene expression.

In addition, Rps26Bp seems to down-regulate the *myc*^{3}-Rps26Ap level and accordingly an Δ*rps26B* mutant results in more *myc*^{3}-Rps26A protein (Figure 4B, lane 3). Therefore, the Rps26Ap seems to play an important role for the formation of both proteins *myc*^{3}*-RPS26A* and *RPS26B* (Figure 4B, lanes 2 + 6), whereas the presence of high amount of Rps26Bp seems to down-regulate the Rps26A protein level (Figure 4B, lanes 3 + 6), but stimulates the *RPS26B* expression (Figure 4B, lane 5).

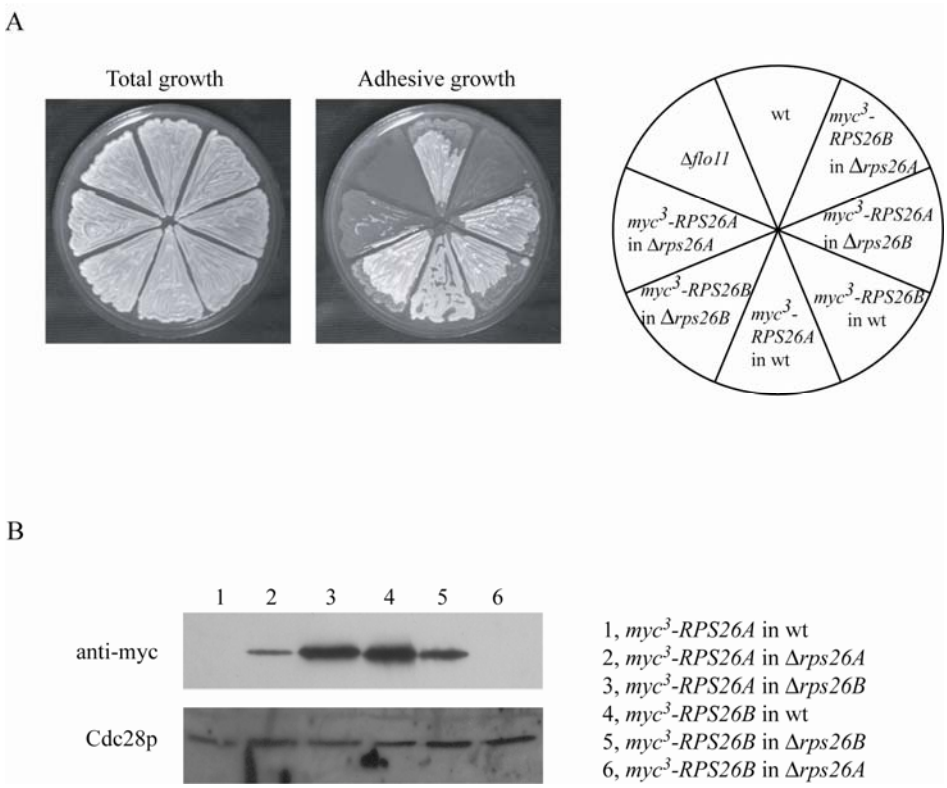

Figure 4. Wash test assay and expression analysis of yeast strains expressing additional *myc*^{3}*-RPS26A* and *myc*^{3}*-RPS26B* in yeast. A. Strains to be tested were patched on YPD medium, visualized after five days of growth (Total growth), washed under a stream of water, and were photographed again to document remaining cells on the agar surface (Adhesive growth). Haploid yeast strains used were RH3176 (*myc*^{3}*-RPS26A* in wt), RH3177 (*myc*^{3}*-RPS26A* in Δ*rps26A*), RH3178 (*myc*^{3}*-RPS26A* in Δ*rps26B*), RH3179 (*myc*^{3}*-RPS26B* in wt), RH3181 (*myc*^{3}*-RPS26B* in Δ*rps26B*) and RH3180 (*myc*^{3}*-RPS26B* in Δ*rps26A*), which all were expressing *myc*^{3}*-RPS26A* or *myc*^{3}*-RPS26B* from behind the original promoter and chromosomally integrated at the *URA3* gene locus. As control wildtype strain RH2584 (wt) and Δ*flo11* strain RH2662 were taken. **B.** Protein extracts were prepared from haploid yeast strains RH3176 (*myc*^{3}*-RPS26A* in wt), RH3177 (*myc*^{3}*-RPS26A* in Δ*rps26A*), RH3178 (*myc*^{3}*-RPS26A* in Δ*rps26B*), RH3179 (*myc*^{3}*-RPS26B* in wt), RH3181 (*myc*^{3}*-RPS26B* in Δ*rps26B*) and RH3180 (*myc*^{3}*-RPS26B* in Δ*rps26A*) which all were expressing *myc*^{3}*-RPS26A* or *myc*^{3}*-RPS26B* from behind the original promoter and chromosomally integrated at the *URA3* gene locus. Levels of *myc*^{3}-Rps26Ap and *myc*^{3}-Rps26Bp were determined by Western blot analysis using an anti-*myc* monoclonal antibody. As internal control, expression levels of Cdc28p were measured within the same extracts using an anti-Cdc28p polyclonal antibody (bottom).

In summary, our data further support that regulation of *RPS26A* and *RPS26B* gene expression is strongly regulated on the translational level. We found a mutual interference between the two isogenic proteins Rps26Ap and Rps26Bp, whereas the exact mechanism is yet unclear. Both *RPS26* gene products are involved in a fine-tuned regulatory system consisting of both proteins.

Discussion

Haploid adhesive and diploid pseudohyphal growth require the formation and expression of *FLO11* mRNA (Lo and Dranginis, 1998; Palecek *et al.*, 2000). In this work we found out that a defect in the *RPS26A* gene has a minor affect on *FLO11* mRNA formation but drastically impairs the translation of the *FLO11* reporter constructs. The effect of a mutation in *RPS26B* on *FLO11* mRNA translation is not sufficient to result in a morphological phenotype. Therefore, the *lacZ*-data give a hint for a translational regulation of *FLO11* through Rps26p. It will be interesting to explore the exact role of the *RPS26* product in translation and to find a mechanistically explanation for the morphological phenotype in Δ*rps26A* cells.

Beside some few exceptions like e.g. *FLO11* (Rupp *et al.*, 1999), yeast promoters seem to be relatively short (Mewes *et al.*, 1997). Both *RPS26* genes require relatively large regions upstream of the ATG start codon for full expression. *RPS26A* requires sequences up to position -750 relatively to the ATG transcriptional start. The *RPS26B* promoter even requires upstream sequences up to -1400, relatively to the ATG transcriptional start. This suggests a very complex regulation of *RPS26B*. It is yet unknown whether this is connected to the fact that the expression of ribosomal proteins exhibits a biphasic response to nutritional changes (Griffioen *et al.*, 1994; Griffioen *et al.*, 1996).

Our expression studies of the two isogenes revealed a complex interdependent regulation, which primarily functions on the post-transcriptional level. Beside different promoter activities, we found an auto-regulation for *RPS26A*, which is negatively affecting its own expression and an auto-regulatory mechanism for *RPS26B*, which is positively affecting its own expression. Such auto-regulations have been described for the expression of ribosomal proteins as *e.g.* Rps14Bp of *S. cerevisiae*. Rps14Ap represses the translation of Rps14Bp by binding to the *RPS14B* pre-mRNA (Fewell and Woolford, 1999). We assume that similar mechanisms play a role in the regulation of *RPS26A* and *RPS26B* expression.

Rps26Bp seems to have an important regulatory role in the wildtype yeast to control Rps26 protein levels in the cell. The protein might be the fine-tuning regulatory protein that enhances translation efficiency. This does not seem to be a feature of the protein itself but of regulatory signals presumably within the transcribed region. In contrast, the Rps26Ap seems to be the protein required for translation, which is normally present in high amounts. Using the *FLO11* expression as one specific translational target, it could be demonstrated that Δ*rps26A* yeast mutant strains fail in translating this transcript. However, there might be additional transcripts, which are not properly translated in such a mutant strain.

Although an expression analysis of Rps26Ap and Rps26Bp protein levels was difficult, due to the fact that the addition of various tags to either the C-terminal or the N-terminal end of the proteins resulted in improper functions, we were able to deduce some interesting features of the regulation mechanisms of *RPS26A* and *RPS26B*, which are summarised in a model in Figure 5.

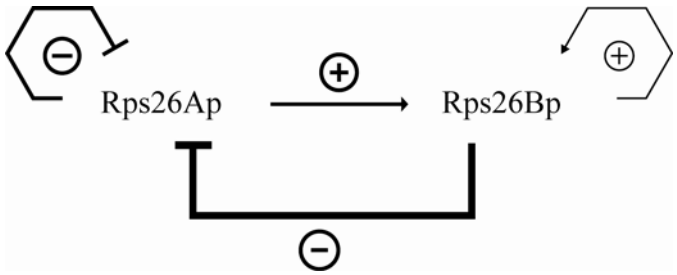

Figure 5. Regulation model of expression levels and translational regulation mechanisms of *rps26A*Δ and *rps26B*Δ yeast mutant strains. A model of the putative activation (+) and repression (-) mechanisms of the two Rps26 proteins is given. Stronger activation or repression as well as stronger feedback inhibition is given by stronger arrows.

This model is not completely consistent with the data from the *lacZ* data given in Figure 3. With the *lacZ*-reporter construct, only the initiation of translation could be measured, whereas determination of protein level reflects also stability and degradation of proteins, which seems to be an important role in the process of

reciprocal regulation of the two isogenes. Therefore, we propose that the Rps26 protein level, which is crucial for exhibiting adherence or filamentous growth, is regulated by a subtle translational mechanism based on different untranslated regions in the transcribed parts of *RPS26A* and *RPS26B*. Our data are in agreement with the following: Rps26Ap stimulates translation of *RPS26B* but reduces its own translation, while Rps26Bp activates its own translation and reduces the translation of the *RPS26A* mRNA. This suggests a more important regulatory role of Rps26Bp in comparison to Rps26Ap, whereas Rps26A is more important for the adhesive phenotype. However, the subtle translational control is only a fine-tuning because the translational control is counteracted by significant differences in the transcription of both genes, where the *RPS26A* promoter is significantly more efficient than is the *RPS26B* promoter. Since a certain lower limit of Rps26 protein is crucial for the developmental function, the higher transcription of *RPS26A* in comparison to *RPS26B* overrules the subtle translational crosstalk between both genes, resulting in the observed phenotypes. This study therefore provides interesting insights into the complex regulatory network connecting two isogenes, which have to be expressed to a basic level to provide competence for development.

References

Abovich, N., Gritz, L., Tung, L., and Rosbash, M. (1985) Effect of *RP51* gene dosage alterations on ribosome synthesis in *Saccharomyces cerevisiae*. *Mol Cell Biol* **5**: 3429-3435.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248-254.

Braus, G.H., Grundmann, O., Brückner, S., and Mösch, H.U. (2003) Amino acid starvation and Gcn4p regulate adhesive growth and *FLO11* gene expression in *Saccharomyces cerevisiae*. *Mol Biol Cell* **14**: 4272-4284.

Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T., and Ramakrishnan, V. (2000) Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* **407**: 340-348.

Cross, F.R., and Tinkelenberg, A.H. (1991) A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. *Cell* **65**: 875-883.

Fewell, S.W., and Woolford, J.L., Jr. (1999) Ribosomal protein S14 of *Saccharomyces cerevisiae* regulates its expression by binding to RPS14B pre-mRNA and to 18S rRNA. *Mol Cell Biol* **19**: 826-834.

Gomez-Lorenzo, M.G., Spahn, C.M., Agrawal, R.K., Grassucci, R.A., Penczek, P., Chakraburtty, K., Ballesta, J.P., Lavandera, J.L., Garcia-Bustos, J.F., and Frank, J. (2000) Three-dimensional cryo-electron microscopy localization of EF2 in the *Saccharomyces cerevisiae* 80S ribosome at 17.5 A resolution. *EMBO J* **19**: 2710-2718.

Green, R., and Noller, H.F. (1997) Ribosomes and translation. *Annu Rev Biochem* **66**: 679-716.

Griffioen, G., Mager, W.H., and Planta, R.J. (1994) Nutritional upshift response of ribosomal protein gene transcription in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* **123**: 137-144.

Griffioen, G., Laan, R.J., Mager, W.H., and Planta, R.J. (1996) Ribosomal protein gene transcription in *Saccharomyces cerevisiae* shows a biphasic response to nutritional changes. *Microbiology* **142**: 2279-2287.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J Bacteriol* **153**: 163-168.

Koc, E.C., Burkhart, W., Blackburn, K., Moseley, A., Koc, H., and Spremulli, L.L. (2000) A proteomics approach to the identification of mammalian mitochondrial small subunit ribosomal proteins. *J Biol Chem* **275**: 32585-32591.

Lambrechts, M.G., Bauer, F.F., Marmur, J., and Pretorius, I.S. (1996) Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. *Proc Natl Acad Sci U S A* **93**: 8419-8424.

Lecompte, O., Ripp, R., Thierry, J.C., Moras, D., and Poch, O. (2002) Comparative analysis of ribosomal proteins in complete genomes: an example of reductive evolution at the domain scale. *Nucleic Acids Res* **30**: 5382-5390.

Lo, W.S., and Dranginis, A.M. (1998) The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol Biol Cell* **9**: 161-171.

Mager, W.H., Planta, R.J., Ballesta, J.G., Lee, J.C., Mizuta, K., Suzuki, K., Warner, J.R., and Woolford, J. (1997) A new nomenclature for the cytoplasmic ribosomal proteins of *Saccharomyces cerevisiae*. *Nucleic Acids Res* **25**: 4872-4875.

Mewes, H.W., Albermann, K., Bahr, M., Frishman, D., Gleissner, A., Hani, J., Heumann, K., Kleine, K., Maierl, A., Oliver, S.G., Pfeiffer, F., and Zollner, A. (1997) Overview of the yeast genome. *Nature* **387**: 7-65.

Miozzari, G., Niederberger, P., and Hutter, R. (1978) Tryptophan biosynthesis in *Saccharomyces cerevisiae*: control of the flux through the pathway. *J Bacteriol* **134**: 48-59.

Myers, A.M., Tzagoloff, A., Kinney, D.M., and Lusty, C.J. (1986) Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. *Gene* **45**: 299-310.

Palecek, S.P., Parikh, A.S., and Kron, S.J. (2000) Genetic analysis reveals that *FLO11* upregulation and cell polarization independently regulate invasive growth in *Saccharomyces cerevisiae*. *Genetics* **156**: 1005-1023.

Planta, R.J., and Mager, W.H. (1998) The list of cytoplasmic ribosomal proteins of *Saccharomyces cerevisiae*. *Yeast* **14**: 471-477.

Roberts, R.L., and Fink, G.R. (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev* **8**: 2974-2985.

Rose, M., and Botstein, D. (1983) Construction and use of gene fusions to *lacZ* (beta-galactosidase) that are expressed in yeast. *Methods Enzymol* **101**: 167-180.

Rupp, S., Summers, E., Lo, H.J., Madhani, H., and Fink, G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene. *EMBO J* **18**: 1257-1269.

Sikorski, R.S., and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19-27.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**: 503-517.

Spahn, C.M., Kieft, J.S., Grassucci, R.A., Penczek, P.A., Zhou, K., Doudna, J.A., and Frank, J. (2001) Hepatitis C virus IRES RNA-induced changes in the conformation of the 40s ribosomal subunit. *Science* **291**: 1959-1962.

Strittmatter, A.W. (2003) Transcriptional Regulation and Differentiation in *Saccharomyces* and *Aspergillus*: *jlbA*, *RPS26*, and *ARO3/4*. *Dissertation*.

Surana, U., Amon, A., Dowzer, C., McGrew, J., Byers, B., and Nasmyth, K. (1993) Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J* **12**: 1969-1978.

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