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Proteins that genetically interact with the *Saccharomyces cerevisiae* transcription factor Gal11p emphasize its role in the initiation-elongation transition

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**Abstract** The *GAL11* gene encodes a transcription factor that is a component of the SRB/Mediator subcomplex of the RNA polymerase II holoenzyme in the yeast *Saccharomyces cerevisiae*. In agreement with this biochemical characterization, Gal11p has been found to be required for optimal production of mRNA from many yeast promoters, and recessive mutations in *GAL11* have been shown to cause pleiotropic defects. Despite this progress, the role of Gal11p in gene regulation remains largely unknown. In a multicopy suppressor analysis of a *gal11Δ* mutation we have identified genes encoding proteins that are part of, or can interact with, the RNA polymerase II transcription complex, as well as factors involved in cell cycle regulation. Among the suppressors that are clearly related to the transcriptional apparatus, Gal11p genetically interacts with components of the SRB/Mediator complex, as well as with factors such as TFIIE and TFIIH that are required for promoter clearance and transcription elongation by RNA polymerase II. These findings, taken together with published results of biochemical and genetic analyses, suggest a role for Gal11p at the interface between the SRB/Mediator complex and the general transcription factors TFIIE and TFIIH, which modulate, via phosphorylation of the CTD, the activity of the RNA polymerase II during the transition between initiation and elongation.

**Keywords** Multicopy suppressors · *GAL11* · RNA polymerase II holoenzyme · Transcription initiation · Transcription elongation

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**Introduction**

A large body of evidence suggests the existence in vivo of a multiprotein transcription complex called the RNA polymerase II (RNA pol II) holoenzyme. This complex was described as being composed of the core RNA pol II, some of the general transcription factors (GTFs), and the SRB/Mediator and the Swi/Snf sub-complexes (Greenblatt 1997). This large multisubunit protein complex is believed to be the form of the RNA polymerase II activity that is recruited to promoters via interaction with DNA-binding transcriptional activators to activate otherwise silent genes (Barberis et al. 1995; Ptashne and Gann 1997; Barberis and Gaudreau 1998).

The present work focuses on the role of the yeast transcription factor Gal11p, which is part of the SRB/Mediator sub-complex of the RNA pol II holoenzyme (Myers et al. 1998). Gal11p was initially identified as a protein required for efficient galactose utilization in yeast (Suzuki et al. 1988). However, later experiments have shown that removal of Gal11p from the yeast cell by deletion of the *GAL11* locus leads to pleiotropic phenotypes, most of which probably result from a general decrease in mRNA production. Indeed, in *gal11Δ* cells the ability of several activators to stimulate transcription is impaired, and the levels of mRNA can drop to 20–50% relative to those in Gal11p+ cells (Barberis et al. 1995; Sussel et al. 1995; Sakurai et al. 1996a).

Interest in Gal11p has been further aroused by the discovery that a variant of this protein called Gal11pp, which bears a single amino acid substitution, is able to turn the otherwise silent DNA-binding activity of Gal4p into a strong activator (Barberis et al. 1995). The fact that a simple contact between a DNA-binding protein and a single component of the RNA pol II holoenzyme suffices for gene activation has provided strong support for the so-called “recruitment model” for the mechanism of transcription activation (Ptashne and Gann 1997; Barberis and Gaudreau 1998). This finding is also consistent...
with a role for Gal11p as a potential partner for transcriptional activators (Lee et al. 1999).

The function of Gal11p within the SRB/Mediator sub-complex is unknown. However, biochemical assays have shown that, in addition to being part of the holoenzyme, Gal11p can establish interactions with proteins outside the SRB/Mediator complex, such as TFIIIE (Sakurai and Fukasawa 1998). This pattern of multiple interactions described for Gal11p suggests that this protein may constitute one of the docking points within the holoenzyme for transcriptional activators and for general transcription factors such as TFIIIE, which are not integral components of the holoenzyme.

Previous attempts to identify proteins that genetically interact with Gal11p led to the isolation of the SGEl gene product (Amakasu et al. 1993), a member of the major facilitator superfamily of multidrug resistance proteins (Jacquot et al. 1997). In their original work, Amakasu et al. (1993) performed a genetic suppression screen based on the inability of a gal11 deletion mutant strain (gal11Δ) to grow on ethidium bromide-containing plates supplemented with galactose as the sole carbon source. In the present work, we devised a different genetic selection strategy based on the observation that removal of the GAL11 gene from the yeast genome leads to a conditional lethal phenotype which consists in growth arrest of the mutant gal11Δ cells when shifted to the non-permissive temperature of 37°C (our unpublished observations; Sussel et al. 1995). We have exploited this growth-arrest phenotype as a suitable selection system for a multicopy suppressor screen of a yeast genomic library. To facilitate the isolation of suppressor clones encoding proteins more directly implicated in Gal11p-dependent transcription, all clones that tested positive in the first selection step were further assayed for their ability to restore impaired transcription of a reporter gene as well as growth on galactose.

Our screening experiments resulted in the isolation of multicopy suppressor clones encoding proteins that could be assigned to various functional categories. The list of suppressors includes proteins that are part of, or may interact with, the RNA pol II transcription complex and key players in important cellular processes such as cell cycle regulation. The identification of proteins involved in gene regulation as multicopy suppressors of gal11Δ phenotypes, taken together with the results of additional genetic and biochemical assays, highlight a positive role for Gal11p in mediating the transition between the initiation and the elongation phase of transcription.

### Materials and methods

#### Yeast media, plasmids and strains

Yeast genetic techniques and media were as described (Kaiser et al. 1994). Yeast transformation was performed by the lithium acetate procedure (Gietz et al. 1995). Plasmid pBL7 is a 2μ-based plasmid containing an expression cassette, made up of the full-length GAL11 gene together with its natural promoter and terminator, and URA3 as the selectable marker. Plasmid pBL9 (HIS3) is an ARS/CEN-based vector that contains the Gal11p expression cassette described for pBL7. The expression plasmids pP273 (LEU2), encoding an N-terminally myc-tagged version of Gal11p; pSO23 (LEU2), encoding Gal11p without the myc tag, and pP224 (TRP1), used to express N-terminally HA-tagged versions of different putative Gal11p interaction partners, were used in co-immunoprecipitation experiments (Barberis et al. 1995). The integrative reporter construct pDE193 (obtained from Dr. D. Escher) is a HIS3-based plasmid containing three multimerized copies of the binding consensus sequence for LexA upstream of a GAL11 minimal promoter and a lacZ reporter. The integrative reporter construct pDE103 (also from Dr. D. Escher) contains four copies of the upstream activating sequences for the transcriptional activator Gal4p (UASr) fused to a GAL11 minimal promoter region driving the expression of a lacZ reporter. For both integrative plasmids, linearization with XhoI allows integration in the 5'UTR region of the HIS3 gene.

The gal11 deletion mutant strain BLY0 (MATa, lys2-200, ura3-52, HIS3::pDE103, leu2-1, trp1-63, gal11:::TRP1) carries both a disrupted chromosomal copy of GAL11 and an integrated copy of the reporter construct pDE103 at the HIS3 locus, which makes this gal11Δ strain suitable for monitoring levels of galactose-inducible transcription. BLY0 was used here to screen for the multicopy suppressors. Strain JPY10ex3 (MATa, lys2-200, ura3-52, HIS3::pDE193, leu2-1, trp1-63, gal11:::LYS2), which carries both a disrupted chromosomal copy of GAL11 and an integrated copy of the reporter construct pDE193 at the HIS3 locus, was used both for co-immunoprecipitation experiments and Lex-mediated transcription assays. Strain JPY10 (MATa, lys2-200, ura3-52, URA3::pRY131, his3-200, leu2-1, trp1-63, gal11:::LYS2) is isogenic to BLY0, the only difference being that the UASr-lacZ reporter gene pRY131 is integrated at the URA3 locus instead of at the HIS3 locus.

#### Yeast genomic DNA library

We obtained a library of S. cerevisiae genomic DNA cloned in YEp352 containing a URA3 marker from Drs. M. Aebi and S. te Heesen (Microbiology Institute of the ETH Zurich, Switzerland; Fleischmann et al. 1996). Inserts are derived from a partial Sac3AI digest of wild-type yeast genomic DNA. Restriction analysis of library plasmid DNA with XbaI + KpnI gave an average insert size of ~ 5 kb.

#### Multicopy suppressor screen

A known number of BLY0 cells (gal11Δ) transformed with the yeast genomic library was plated on SD-ura-trp medium and incubated at 37°C until temperature-resistant colonies appeared. To provide positive and negative controls for complementation of the Gal11 phenotypes, pBL7 and its (empty) parental plasmid YEp352 (Gietz and Sugino 1988) were also included in the transformation experiment and analyzed for growth and transcription levels during all the subsequent screening steps. To check for the transformation efficiency, aliquots of transformed cell suspensions corresponding to known numbers of cells were plated and incubated at 30°C. All temperature resistant colonies were successively analyzed following the complete selection procedure illustrated in Fig. 1. The genomic clones that could reproducibly complement the gal11Δ phenotypes after reintroduction into BLY0 were regarded as true positives.

### β-Galactosidase assay in liquid culture and on X-gal plates

β-Galactosidase activity in liquid cultures was assayed as previously described (Rose and Botstein 1983). Experiments were done in triplicate and for all clones examined standard deviations were