Regulation of Transcription by the Ubiquitin-specific Protease Ubp14p

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ABSTRACT

The ubiquitin-proteasome system (UPS) is intimately involved in the regulation of gene transcription. Both ubiquitin and the proteasome can carry out a diverse range of proteolytic and non-proteolytic activities, making it well-poised in controlling the activities, levels and distribution of transcriptional machinery components. A level of regulation in the UPS in the yeast *Saccharomyces cerevisiae* is provided by ubiquitin-specific proteases (UBPs), which are involved in the removal and processing of ubiquitin. These proteases have been found to positively regulate or inhibit proteolysis. In this study, the deletion of *UBP14* causes defects in glucose repression (i.e. glucose derepression phenotype), indicating a role of Ubp14p in regulating transcription. Preliminary findings suggest that this glucose derepression phenotype of the Δ*UBP14* strain is linked to the stabilisation of the transcriptional activator Gal4p and that Ubp14p serves to either positively regulate its degradation or remove its monoubiquitin signal that is necessary for stable promoter binding.

INTRODUCTION

Ubiquitin-specific proteases are the largest class of deubiquitinating enzymes in *S. cerevisiae*. These UBPs mainly cleave ubiquitin from ubiquitinated protein substrates, although Ubp14p has been found to cleave ubiquitin oligomers (Hochstrasser, 1996; Amerik et al., 1997). Studies performed to understand the roles of UBPs in the regulation of protein degradation or modification have presented conflicting evidence: UBPs can have either positive or inhibitory effects on degradation. For example, Ubp3p deubiquitinates certain proteins which stabilises them from proteasomal degradation (Brew et al., 2002), but Ubp3p also has a seemingly opposing role as it is required for the selective degradation of mature ribosomes by autophagy (Kraft et al., 2008). Additionally, Ubp14p was shown to disassemble unanchored ubiquitin oligomers *in vitro* and was proposed to facilitate degradation by preventing these chains from competitively inhibiting the binding of polyubiquitinated substrates to the proteasome (Amerik et al., 1997). Deletion of *UBP14* causes the accumulation of these unanchored ubiquitin chains, causing inhibition of degradation. In a later study by Eisele et al. (2006), it was found that the accumulation of these chains does not inhibit the degradation of all proteins in general, but inhibits the degradation of only certain proteins, indicating that the inhibition is pathway specific. Collectively taken, these findings indicate the complexity of the UBPs in protein degradation.

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The UPS is closely connected to the regulation of gene transcription. One way in which ubiquitination affects transcription is by the regulation of protein levels of transcriptional activators and repressors. The polyubiquitination of transcriptional activators results in their proteolysis, and this can prevent activation, terminate transcription or even allow for the progress of translation (Muratani and Tansey, 2003). In addition to their roles in proteolysis, the UPS has also been found to have non-proteolytic roles in regulating transcription. For example, the monoubiquitination of the histones H2A and H2B is associated with actively transcribed genes and is believed not to be linked to their degradation (Zhang, 2003).

Furthermore, Ubp8p has been found to deubiquitinate histone H2B (Henry et al, 2003). Ubp3p has also been found to deubiquitinate RNA polymerase II and prevent its degradation (Kvint et al, 2008). These findings suggest that ubiquitination, as well as deubiquitination by UBPs are intimately involved in DNA biology.

One well-characterised paradigm of transcriptional regulation in yeast is the \textit{GAL} system (Traven et al., 2006). The \textit{GAL} system is under dual control – in galactose, transcription of the \textit{GAL} genes is rapidly induced by the activator Gal4p; in glucose, the \textit{GAL} genes are repressed by the repressors Mig1p and Mig2p. A \textit{UBP14} deletion strain was found to exhibit glucose derepression, resulting in transcription from the \textit{GAL1} promoter even in the absence of galactose as the inducer. The aim of the project is to study the role of Ubp14p in the regulation of transcription, as well as to identify and characterise its potential substrates. The project also aims to identify suppressors of the glucose derepression phenotype of the \textit{UBP14} deletion strain.

**MATERIALS & METHODS**

**Phenotype assays**

The yeast strains used in the phenotype assays were from the background BY4741 (\textit{MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0}). To test the phenotype of the yeast strains, a series of 10-fold dilutions from $10^0$ to $10^5$ was performed in sterile water. 5 µl of each dilution was spotted onto agar plates containing selective media and incubated at 28°C for the required number of days.

**Cycloheximide chase assays and western blot analysis**

Cycloheximide is a potent inhibitor of protein biosynthesis in eukaryotic organisms (Hampsey, 1997). To determine the stabilities of Mig1p-myc9 and Mig2p-myc9 in glucose, the required yeast strains were inoculated in 10ml selective media and incubated at 28°C to OD$_{600nm}$ ~ 1.0. Cycloheximide was added to the cultures and aliquots were collected at 1h intervals. The cell pellets were loaded onto 8% polyacrylamide gels. The proteins were transferred to a nitrocellulose membrane and probed with an α-myc antibody.

**RESULTS & DISCUSSION**

**Phenotyping of UBP deletion strains**

From Figure 1, while the wildtype strain has a lack of growth on the U- plate and exhibits glucose repression as expected, the ΔUBP14 strain has a strong glucose derepression phenotype as it consistently displayed growth comparable to the ΔMIG2 strain in three separate assays. Other UBP deletion strains (ΔUBP1, ΔUBP3, ΔUBP6, ΔUBP7, ΔUBP8, ΔUBP10 and ΔUBP13) also exhibit glucose derepression, but to a smaller extent than the ΔUBP14 strain. Thus, the project has chosen to focus on the role of \textit{UBP14} in glucose repression.
Stability of Mig1p-myc9 and Mig2p-myc9 in glucose

A possible hypothesis for the glucose derepression phenotype is that in glucose, Ubp14p deubiquitinates a repressor at the GAL1 promoter and stabilises it by preventing its proteasomal degradation. In the ΔUBP14 strain, the repressor is not deubiquitinated and is destabilised, resulting in transcription from the GAL1 promoter even in the absence of galactose as the inducer. As Mig1p and Mig2p are known to be involved in glucose repression, a cycloheximide chase assay was performed on Mig1p-myc9 and Mig2p-myc9 to determine if the glucose derepression phenotype of the ΔUBP14 strain was due to their decreased stability.

From Figure 2, Mig1p-myc9 remained relatively stable in both wildtype and ΔUBP14 strains. However, Mig2p-myc9 was degraded more rapidly in the wildtype strain than in the ΔUBP14 strain. These results indicate that the glucose derepression of the ΔUBP14 strain is not due to the destabilisation of Mig1p and Mig2p as both remain stable. Rather, they point at an alternative mechanism for the transcription at the GAL1 promoter in the absence of galactose.

Deletion of GAL4 suppresses the glucose derepression phenotype of the ΔUBP14 strain

An alternative hypothesis is that in glucose, Ubp14p deubiquitinates the activator Gal4p at the GAL1 promoter as part of ubiquitin-dependent degradation, resulting in its destabilisation. In
the ΔUBP14 strain, Gal4p is stabilised and activates transcription at the \textit{GAL1} promoter even in the absence of galactose as the inducer. This is supported by studies on other UBPs, where their deletions lead to the stabilisation of certain proteins. Another hypothesis is that in glucose, Ubp14p removes the monoubiquitin from Ga14p, which has been shown to be essential for promoter binding \textit{in vivo} (Archer et al, 2008). In the ΔUBP14 strain, Ga14p remains monoubiquitinated and binds stably to the \textit{GAL1} promoter, activating transcription.

In both hypotheses above, Gal4p is believed to be stabilised in glucose, resulting in the glucose derepression phenotype of the ΔUBP14 strain. To test these hypotheses, double deletion strains of \textit{UBP14} and \textit{GAL4} were generated and tested for their phenotype on glucose plates.

![Figure 3. Suppression of the glucose derepression phenotype in the double deletion strains of \textit{GAL4} and \textit{UBP14}. Tenfold serial dilutions of the yeast strains were spotted onto W- glucose (control) and UW- glucose.](image)

It can be inferred that the deletion of \textit{GAL4} suppresses the glucose derepression phenotype of the ΔUBP14 strain. This indicates that the glucose derepression is due to Gal4p being stabilised and concurs with both hypotheses mentioned above.

**REFERENCES**


