Role of the yeast Snf1 protein kinase in invasive growth

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Abstract

The sucrose non-fermenting 1 (Snf1) protein kinase of *Saccharomyces cerevisiae* is important for transcriptional, metabolic and developmental responses to glucose limitation. Here we discuss the role of the Snf1 kinase in regulating filamentous invasive growth. Haploid invasive growth occurs in response to glucose limitation and requires *FLO11*, a gene encoding a cell-surface adhesin. Snf1 regulates transcription of *FLO11* by antagonizing the function of two repressors, Nrg1 and Nrg2. Snf1 and the Nrg repressors also affect diploid pseudohyphal differentiation, which is a response to nitrogen limitation, suggesting an unexpected signalling role for the Snf1 kinase.

Introduction

The sucrose non-fermenting 1 (Snf1)/AMP-activated protein kinase (AMPK) family of protein kinases is highly conserved in fungi, plants and animals and has broad roles in transcriptional and metabolic responses to cellular stress [1,2]. In the budding yeast *Saccharomyces cerevisiae*, the Snf1 kinase is primarily known for its role in adaptation to glucose limitation, but it has also been implicated in other stress responses [3,4]. The function of Snf1 in transcriptional and metabolic regulation has been extensively examined, as has its role in meiosis and sporulation. More recently, it has become apparent that Snf1 is also required for other developmental processes, namely filamentous invasive growth in haploids and diploids [5,6].

Haploid invasive growth is a cell-type-specific developmental process in which cells assume an elongated morphology, alter their budding pattern and invade the agar [7–10]. Invasive growth occurs in response to carbon limitation signals and depends on the Snf1 kinase [5]. One important step in this process is the transcriptional activation of the *FLO11* gene, which encodes a cell-surface glycoprotein that functions in cell-cell adhesion and adherence to surfaces [11–14]. *FLO11* has a large promoter that is regulated by the Kss1 mitogen-activated protein kinase cascade, which works through the activator Ste12-Tec1, and by cAMP-dependent protein kinase, which controls the transcriptional repressor Sfl1 and the activator Flo8 [7,13,15–18].

We here discuss the role of the Snf1 kinase and two repressor proteins, Nrg1 and Nrg2, in regulating *FLO11* transcription in response to glucose depletion. We also discuss evidence that Snf1 and the Nrg repressors affect diploid pseudohyphal differentiation, another Flo11-dependent process, but one that is regulated by nitrogen, not glucose. The genetic and physical relationships between Snf1 and Nrg proteins suggest a model in which Snf1 positively regulates Flo11-dependent developmental processes by antagonizing Nrg-mediated repression of *FLO11*.

Snf1 kinase regulates transcription of *FLO11* in response to glucose signals

Analysis of RNA levels showed that the Snf1 kinase regulates *FLO11* transcription in response to glucose limitation [6] (Figure 1). When wild-type cells of the invasive haploid strain Σ1278b were shifted from high to low (0.05%) glucose, *FLO11* RNA levels increased dramatically. When similarly shifted, *snf1* mutant cells derepressed *FLO11* weakly, consistent with their reduced ability to invade agar. These findings indicate that Snf1 is required for derepression of *FLO11*. In contrast, constitutive up-regulation of Snf1 activity in a *reg1* mutant led to elevated *FLO11* expression and enhanced invasive growth [6]; *Reg1* is a targeting subunit that directs protein phosphatase 1 to inhibit Snf1 [19–22]. These findings indicate that Snf1-dependent regulation of *FLO11* is important in the control of haploid invasive growth in response to glucose signals. Further studies will be required to determine whether Snf1 also regulates other, as yet unidentified, targets that contribute to invasive growth.

Snf1 counteracts repression by the Nrg1 and Nrg2 proteins

The zinc-finger repressor proteins Nrg1 and Nrg2 interact physically with Snf1 [23] and mediate glucose repression of various Snf1-dependent genes [23–25]. Several lines of evidence support the view that Snf1 antagonizes Nrg1- and Nrg2-mediated repression of the *FLO11* promoter [6]. First,
Snf1 kinase regulates FLO11 expression

Wild-type (WT), snf1 and reg1 strains were grown to mid-logarithmic phase at 25°C in rich medium containing 2% glucose (glucose-repressed, R). Wild-type and snf1 cultures were also shifted to rich medium with 0.05% glucose for 135 min (derepressed, D). Total RNAs were prepared and fractionated on a 0.8% agarose/formaldehyde gel, and FLO11 RNA was detected by Northern blot analysis with a probe prepared from a PCR product containing the first 208 codons of FLO11 [6]. The gel was stained to visualize the rRNA and confirm that all lanes were equivalently loaded.

It is also possible that the Nrg repressors control other genes besides FLO11 that are important for invasive growth, and it is possible that Snf1 affects FLO11 expression by other mechanisms that do not involve Nrg proteins. The Snf1 kinase has multiple regulatory targets in the cell that mediate different responses to glucose limitation [4]. These targets include both transcriptional activators and repressors, and Snf1 conceivably could also regulate activators of FLO11.

Figure 1 | Snf1 kinase regulates FLO11 expression

Figure 2 | Model for the roles of Snf1 kinase in invasive growth

(A) In haploid invasive growth, the Snf1 kinase is activated in response to glucose limitation and relieves Nrg-mediated repression of FLO11. It is also possible that Snf1 affects FLO11 by other Nrg-independent mechanisms. (B) In diploid pseudohyphal differentiation, we propose that the Snf1 kinase is activated under conditions of nitrogen limitation and up-regulates FLO11; however, other possibilities are not excluded.

Snf1 and the Nrg repressors regulate diploid pseudohyphal development

Diploid pseudohyphal differentiation is in many respects similar to haploid invasive growth [8–10]. Cells assume an elongated morphology, change their budding pattern and generate chains of filamentous-form cells. Moreover, this developmental process depends on FLO11 [11,13,27]. A major difference, however, is that pseudohyphal growth occurs in response to limitation for nitrogen, not glucose. Nonetheless, the Snf1 kinase and the Nrg proteins proved to have roles in pseudohyphal differentiation [6]. A diploid homozygous for the snf1 mutation was defective in the formation of pseudohyphae in response to nitrogen limitation (growth on low-ammonia plates). In contrast, mutation of the NRG genes increased pseudohyphal differentiation and also partially restored pseudohyphal growth in an snf1 mutant diploid (homozygous for snf1 nrg1 nrg2). These findings suggest that Snf1 regulates pseudohyphal differentiation by antagonizing Nrg1- and Nrg2-mediated repression; FLO11 is a likely target, although there is, as yet, no direct evidence to support this idea (Figure 2).

These results suggest, unexpectedly, that the Snf1 kinase plays a role in a response to nitrogen limitation. Although the basal activity of Snf1 may simply be required during pseudohyphal differentiation, it is equally possible that Snf1 activity is induced by low nitrogen. It is already clear that the Snf1 kinase responds to multiple signalling inputs, as two different pathways regulate the activity and localization of Snf1 in response to different carbon source signals [28], and Snf1 could certainly receive yet other nutrient signals.

Conclusion

The Snf1 kinase has roles in cell-type-specific developmental processes that are regulated by distinct nutrient signals: Snf1 is required for haploid invasive growth in response to glucose limitation and for diploid pseudohyphal differentiation in response to nitrogen limitation. The evidence suggests a model in which Snf1 positively regulates Flo11-dependent developmental events by antagonizing Nrg-mediated repression of the FLO11 gene. Although it is possible that Snf1 indirectly antagonizes repression by Nrg1 and Nrg2, the physical interaction between Snf1 and the Nrg proteins [23] suggests direct functional interaction.

The SNF1 and NRG genes are conserved in the fungal pathogen Candida albicans, where the Nrg1 orthologue
represses filamentous growth and expression of adhesin genes [29,30]. Hence, C. albicans Snf1 may have a role in the morphological transition from yeast form to filamentous growth, a process that is essential for pathogenicity.

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References

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