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Construction of yeast strains useful for screening drugs that inhibit glucose uptake and glycolysis

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Abstract

The budding yeast *Saccharomyces cerevisiae* expresses different isoforms of glucose transporters (HXTs) in response to different levels of glucose. Here, we constructed reporter strains in which the nourseothricin (NAT) resistance gene is expressed under the control of the *HXT1*, 2, or 3 promoter. The resulting *HXT-NAT* reporter strains exhibited a strict growth dependence on glucose, and their growth could be easily controlled and optimized by adjusting glucose concentration, demonstrating the value of the reporter strains for studying the molecular basis of differential expression of *HXT* genes, as well as for screening drugs that inhibit glucose uptake and glycolysis.

Keywords

Glycolysis; Glucose uptake; Glucose transporters; Yeast; *HXT-NAT* reporter strains

Cancer cells consume glucose avidly to maintain the high rate of aerobic glycolysis, a phenomenon referred to as the Warburg effect [1]. A characteristic of this phenomenon is increased glucose uptake as a result of overexpression of glucose transporters. The budding yeast *Saccharomyces cerevisiae* also displays aerobic glycolysis and thus consumes glucose vigorously even in the presence of oxygen by upregulating expression of glucose transporters [2]. The yeast has at least 6 glucose transporters (Hxt1-4, Hxt6, and Hxt7) with different affinities for glucose. Expression of *HXT* genes encoding glucose transporters is induced by glucose, but the yeast expresses only the glucose transporters best suited for the amount of glucose available in the environment as discussed below, implicating a correlation between transporter regulation and function [3, 4]. For example, the low affinity transporter gene *HXT1* is expressed only in the presence of high concentrations of glucose; in contrast, expression of high affinity transporter genes such as *HXT2*, *HXT4*, *HXT5*, and *HXT6* is induced by low levels of glucose. It is widely known that expression of Hxt1 transporter in the presence of high levels of glucose leads to a reduction of the concentration of glucose in the culture medium due to glucose uptake by Hxt1, which in turn leads not only to induction of expression of high affinity glucose transporters but also to repression of Hxt1 expression. This regulatory property of glucose transporters hampers the development

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of effective methods to determine differential expression of different *HXT* genes in response to different levels of glucose. Here, we present a novel method to overcome this limitation by constructing yeast reporter strains that express only transporters most appropriate for the given glucose concentration in the presence of the antibiotic nourseothricin (NAT).

We constructed *HXT-NAT* reporter strains that express the *NAT* resistance gene under the control of the *HXT1*, *HXT2*, or *HXT3* promoter by using the PCR-based gene deletion strategy [5, 6]. To do this, the *NAT* ORF was PCR-amplified from the NatMX cassettes [7], and the BY4741 yeast was transformed with the PCR products. To identify transformants in which the *NAT* gene is integrated with the *HXT* gene sequences, genomic DNA was isolated and used as the template in PCR reactions using two primer sets; one primer that anneals within the *NAT* gene and the other primer that anneals to the *HXT* gene locus outside region altered. PCR products of the expected sizes confirmed the homologous integration of the *NAT* gene into *HXT* gene sequences (Fig. 1A).

HXT-NAT reporter strains were spotted on YP (2% bacto-peptone, 1% yeast extract) plate containing different concentrations of glucose (0% - 2%) supplemented with 100 µg/ml NAT-sulfate. The first spot of each row represents a count of 5×10^7 cell/ml, which is diluted 1:10 for each spot thereafter and incubated for 3 days. *HXT-NAT* reporter strains were shown to be susceptible to nourseothricin in the absence of glucose (Glycerol/Ethanol medium), but exhibited resistance to the antibiotic only when glucose is present in the growth medium (Fig. 1B). We found that the growth of the *HXT1-NAT* reporter strain is more easily detected in high rather than low glucose concentration (2% glucose), and that the *HXT2-NAT* reporter strain grows better in low glucose medium such as raffinose-containing medium (raffinose is equivalent to low levels of glucose) than in high glucose medium. The *HXT3-NAT* reporter strain was shown to grow in both low and high glucose media, as reported previously [3]. These results indicate that the growth of the three reporter strains in the presence of nourseothricin is regulated by glucose concentration. Glucose-induction of *HXT* expression requires inactivation of the Rgt1 repressor, a master repressor of *HXT* genes [8, 9]. Rgt1 appears to mediate repression of all *HXT* genes by binding, and recruiting the general corepressor complex Ssn6-Tup1, to the upstream regions of the genes [10, 11]. It remains elusive how Rgt1 is inactivated by low and high glucose signals. In this regard, the system developed in this study would provide a novel, facile, and highly controllable method to study regulatory mechanisms of expression of specific *HXT* genes *in vivo*. As Hxt2 is a high affinity glucose transporter, similar to Hxt4, Hxt6, and Hxt7, in regard that they all are expressed in the presence of low glucose concentration, we constructed only the *HXT2-NAT* reporter strain as a representative of the whole group.

Next, we investigated whether our system is useful for screening drugs that inhibit glucose uptake and glycolysis by examining the effect of the glucose analog 2-deoxy-D-glucose (2-DG) on growth of the *HXT-NAT* reporter strains. 2-DG, a competitive inhibitor of glucose, is transported into cells by glucose transporters but cannot be metabolized. To this end, the *HXT-NAT* reporter strains were tested for growth in glucose plus NAT plates containing different concentrations of 2-DG and incubated for 3 days. The results showed that growth of all the reporter strains tested is significantly inhibited by 2-DG, and that expression of the *HXT1* gene is more sensitive to 2-DG than that of *HXT2* or *HXT3* gene (Fig. 2). These observations are consistent with our previous finding that 2-DG inhibits induction of expression of *HXT* genes [12] and also support the view that expression of different *HXT* genes may be regulated by different mechanisms [2]. The critical components for the glucose induction of *HXT* gene expression are the glucose sensors Rgt2 and Snf3, plasma membrane proteins that sense the presence of extracellular glucose and generate a signal that leads to inactivation of Rgt1 [2, 4]. The glucose sensors are very similar to glucose transporters in their structures but appear to be unable to transport glucose [4]. Therefore,

our results suggest that the *HXT-NAT* reporter strains may be useful for screening drugs that inhibit glucose sensors and/or glucose transporters, as well as components of the glucose signaling pathways involved in regulation of *HXT* gene expression [2].

In conclusion, in this study we constructed several yeast *HXT-NAT* reporter strains that are resistant to the antibiotic nourseothricin only in the presence of glucose. Our results demonstrating that the growth of the reporter strains can be easily controlled by glucose concentration suggest that the reporter strains may serve as useful tools for studying the regulatory mechanism of differential expression of *HXT* genes in response to different glucose concentrations. Increased glucose uptake associated with aerobic glycolysis is a hallmark of cancer and its inhibition is a cancer therapeutic strategy that is being intensively investigated. This phenomenon is also observed in the budding yeast, allowing us to consider the reporter strains useful to screen drugs that inhibit glucose uptake and glycolysis in yeast cells as well as cancer cells.

Acknowledgments

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A

B

Fig. 1.

The *HXT-NAT* reporter strains exhibit a strict growth dependence on glucose. (A) Schematic representation of the construction of the *HXT-NAT* reporter strains. For example, the *HXT1* ORF was replaced with *NAT* ORF, which was PCR-amplified from NatMX cassette [7], by the PCR-based gene deletion method [5, 6]. Expression of the *NAT* gene is under the control of the respective *HXT* promoters. (B) Yeast cells (BY 4741 (*Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δ*)) were spotted on YP (2% bacto-peptone, 1% yeast extract) plates containing 5% glycerol + 2% ethanol (Gly/EtOH), 2% glucose (Glu), or 2% raffinose (Raf) supplemented with 100 μ g/ml NAT-sulfate. The first spot of each row represents a count of 5×10^7 cell/ml, which is diluted 1:10 for each spot thereafter. Cells were incubated for 3 days.

Fig. 2.

2-DG inhibits growth of the *HXT-NAT* reporter strains. Yeast cells (BY 4741 (*Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δ*)) were spotted on YP (2% bacto-peptone, 1% yeast extract) plates containing 2% glucose, 100 µg/ml NAT-sulfate, and different concentrations of 2-DG. The first spot of each row represents a count of 5×10^7 cell/ml, which is diluted 1:10 for each spot thereafter. Cells were incubated for 3 days.