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Review

Does a shift to limited glucose activate checkpoint control in fission yeast?

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ABSTRACT

Here we review cell cycle control in the fission yeast, *Schizosaccharomyces pombe,* in response to an abrupt reduction of glucose concentration in culture media. *S. pombe* arrests cell cycle progression when transferred from media containing 2.0% glucose to media containing 0.1%. After a delay, *S. pombe* resumes cell division at a surprisingly fast rate, comparable to that observed in 2% glucose. We found that a number of genes, including zinc-finger transcription factor Scr1, CaMKK-like protein kinase Ssp1, and glucose transporter Ght5, enable rapid cell division in low glucose. In this article, we examine whether cell cycle checkpoint-like control operates during the delay and after resumption of cell division in limited-glucose. Using microarray analysis and genetic screening, we identified several candidate genes that may be involved in controlling this low-glucose adaptation.

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1. Genetically defined mechanism for adapting to limited glucose in *S. pombe*

Glucose is a fundamental energy source in eukaryotes, and is essential to drive energy-dependent physiological processes, such as cell cycle progression. While the fission yeast, *Schizosaccharomyces pombe*, grows and divides vigorously in laboratory media containing high concentrations of glucose (2–3%, or 111–167 mM), such high glucose concentrations are not always available in natural environments. Indeed, we previously reported that fission yeast can proliferate at essentially normal rates in media containing as little as 0.08% (4.4 mM) glucose, which is equivalent to the blood glucose level in a healthy human before breakfast [1,2]. Two genes, *ssp1*$^+$ and *sds23*$^+$, which encode Ca^{2+}/Calmodulin-dependent kinase kinase (CaMKK)-like protein and an inhibitory regulator of type-2A and 2A-related phosphatases (PP2A and PP6), respectively, were essential for fast proliferation in low glucose [1]. Thus, wild-type (WT) fission yeast apparently possess genetically defined mechanisms for adapting to low glucose. Further reduction of glucose concentration slowed cell cycle progression, and in medium containing only 0.02% (1.1 mM), the cell cycle became stochastic, and cells ultimately entered quiescence [2]. In this article, we summarize our recent findings regarding cellular adaptation to low glucose and discuss cellular mechanisms that may enable cell division in low glucose.

2. Cell cycle delay upon reduction of glucose concentration

When *S. pombe* WT cells growing exponentially in synthetic EMM2 liquid medium containing a high glucose concentration (111 mM) were transferred to low-glucose EMM2 (4.4 mM), cells transiently stopped dividing for the equivalent of one or two generations, before resuming vigorous cell division at a rate similar to that in high glucose (Fig. 1A) (also shown in [1,2]). Consistently, the % septation index, the proportion of cells forming the cell plate (septum) prior to cytokinesis, sharply and transiently decreased to a minimal level within 2 h after the shift to low glucose; however, the % septation index was restored after 4 h (Fig. 1B). The temporary decline of the % septation index suggests that cell cycle progression is briefly arrested or delayed.

To test whether ATP concentration was maintained after the glucose shift, we measured intracellular ATP per cell using the luciferase method [3,4]. Immediately upon the shift to low glucose, the intracellular ATP level rapidly decreased to ~20% of the initial ATP level, and then slowly returned to approximately 50% (Fig. 1B), even when cells resumed vigorous division in low glucose (Fig. 1A). From the result of the ATP measurement and an estimated cell volume, we calculated that the intracellular ATP concentration before the glucose shift was 0.97 mM, which is in good agreement with

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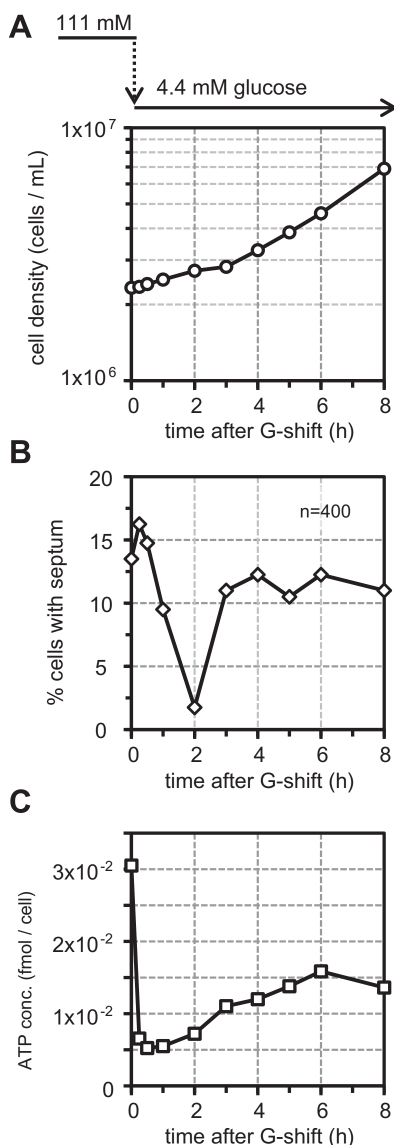

Fig. 1. Cellular responses to reduced extracellular glucose concentration. (A–C) WT fission yeast cells grown in high-glucose (111 mM) EMM2 medium were transferred to low-glucose (4.4 mM) medium and cultivated at 26 °C. Aliquots of the cell culture were taken before (time = 0 h) and after the transfer to low glucose. Cell density (A), % septation index (B), and intracellular ATP concentration (C) were measured. For measurement of the % septation index, glutaraldehyde-fixed cells were stained with Calcofluor White (Sigma–Aldrich, St. Louis, MO, USA), which binds specifically to cell wall and septum, and the proportion of cells having septa was calculated by examining 400 cells. Experiments were done three times at 26 °C, and the septation indices obtained are 14.4 ± 2.5% before the shift, 2.2 ± 0.8% at minimum, 12.0 ± 0.2% after recovery. Intracellular ATP concentration was quantified with a luciferase-mediated luminescence assay using BacTiter-Glo reagent (Promega, Madison, WI, USA), as previously described [4]. Measurements of ATP were done also three times. Concentrations of ATP (100% before the shift) was reduced to the minimum 15.5 ± 2.6% after 15–30 min and restored to 42.9 ± 9.4% after 4–6 h.

previous measurements, 1 mM [5,6]. We presume that during the transient cell cycle arrest after the shift to low-glucose medium, cellular energy metabolism must be greatly altered so that cells can proliferate with a reduced intracellular ATP reserve, possibly requiring a change from glycolysis to respiration. Cellular uptake or utilization of glucose, the main substrate for ATP generation, may temporarily diminish to adapt to limited glucose (Respiration produces about 19× more ATP, so it may be that cells switch to respiration so as to maintain ATP production despite limiting glucose). Alternatively, the shift in energy production systems may simply take time, causing the temporary ATP depletion. In high glucose, ATP production may be excessive, while in low glucose, ATP production and consumption may be balanced.

3. Extensive changes in metabolome profile from high to low glucose

We employed liquid-chromatography mass-spectrometry (LC–MS) to perform quantitative metabolomics on *S. pombe* cell extracts, and identified many metabolites that changed concentration according to glucose concentration [2]. In 2.2 mM glucose, the ATP concentration was about a half that observed in 111 mM glucose. A sharp rise in S-adenosyl methionine (SAM) was observed under low and starved glucose conditions. Consistent with this finding, concentrations of certain free, methylated (mono-, di-, and tri-methylated) amino acids and methylated nucleosides significantly increased. In addition, biosynthesis of ergothioneine and trehalose, both of which are suspected to be stress-responsive compounds, sharply increased under limited glucose. Therefore, we conclude that *S. pombe* cells accumulate anti-stress compounds (anti-oxidants) when dividing rapidly in low glucose, probably due to increased oxidative stress caused by a switch from glycolysis to respiration.

4. Changes in gene transcription after the shift to low glucose

Comprehensive transcriptome analysis using *S. pombe* DNA microarray was conducted before and after the glucose shift. Gene transcription was greatly affected by reduction of the extracellular glucose concentration. WT cells growing exponentially at 26 °C in EMM2 medium containing high (111 mM, 2%) glucose were transferred to low-glucose (4.4 mM, 0.08%) medium, and mRNAs were isolated and analyzed before and at intervals of 1, 2, 3, 4, 6, 12, and 24 h after the transfer. Results are shown in Fig. 2A, B, and C. The cell number stopped increasing for first 4 h after the transfer, and then resumed at a rate similar to that in high-glucose. The glucose concentration remaining in the medium was depleted to ~1.5 mM at 12 h, and was hardly detectable at 24 h (data not shown). Among ~5000 *S. pombe* genes we examined, 1800 gene transcripts showed significant alteration (>100% increase or <50% decrease) in their expression levels after the transfer to low-glucose medium.

According to their expression profiles, these genes were classified into 10 clusters (UP 1–6 and DN 1–4) as shown in Fig. 2A. UP clusters 1–5 contain genes that are up-regulated in the first 1–2 h (~500 genes) while DN 2–4 contain genes that are down-regulated in the first 1–2 h (~600 genes). Expression levels of genes in groups UP6 (312 genes) and DN1 (412 genes) did not change, except 24 h later, when glucose in the medium was nearly 0 mM, implying that changes in expression levels of these genes are related to glucose depletion. Immediately and highly up-regulated transcription (UP 1–4) occurs in 123 genes. Interestingly, four of these (*ght1*$^+$, *ght3*$^+$, *ght4*$^+$, *ght6*$^+$) in UP1, in which transcription levels elevated sharply and then remained high, are closely related and are predicted to encode hexose/glucose transporters. *S. pombe* has eight hexose transporters [7,8]. In addition, genes in cluster UP4 include those related to utilization of carbon sources other than glucose, such as polysaccharides and glycerol, although the medium contained only glucose as a carbon source.

Increased glucose uptake by hexose transporters may promote rapid cell proliferation even in low glucose. Consistent with this notion, we find that one of the putative *S. pombe* hexose transporter genes, *ght5*$^+$ (belonging to UP5), the mRNA level of which is already high before the shift to low glucose and further increases

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