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基礎科学

The RNA-binding protein Whi3 modulates G2/M transition and checkpoint signaling in *Saccharomyces cerevisiae*

■ INTRODUCTION

The eukaryotic cell cycle is regulated by cyclin-dependent kinases (CDKs) and their periodic association with activating subunits called "cyclins". In *Saccharomyces cerevisiae,* Cdc28 is the sole CDK, and it interacts with nine periodically expressed G1 cyclins and B-type cyclins. The B-type cyclins Clb1 and Clb2 accumulate during the transition from G2 to M phase and are essential for mitotic spindle elongation, spindle pole body separation, and mitotic exit. The proper timing and expression of these cyclins are crucial for maintaining genomic stability and coordinating mitotic progression.[1]

RNA-binding proteins (RBPs) play key roles in the control of cell cycle regulators. Recently, our laboratory reported that Puf5, belonging to the Puf family of RBP, positively regulates the expression of the G2/M phase cyclins *CLB1* and *CLB2* by binding to the *IXR1* mRNA.[2]

Another RBP, Whi3, has been implicated in controlling G1/S transition by binding to the *CLN3* mRNA. However, although studies have reported the association of Whi3 with other cyclins, such as G2/M cyclins *CLB1* and *CLB2*, its broader role in their regulation remains unclear.[3]

Thus, the current study investigates the role of Whi3 in the regulation of G2/M phase and checkpoint signaling in *Saccharomyces cerevisiae.*

■ METHODS

1. Strains and media

Saccharomyces cerevisiae W303 was the background strain. DNA manipulations used *E. coli* DH5α, and mutants were generated by PCR-based gene deletion. Standard cultures were grown in YPD (2% glucose, 2% Bacto peptone, 1% yeast extract).

2. Cell cycle synchronization by α-factor block

Yeast cells were pre-cultured overnight in YPD medium at 28℃, and then transferred into a fresh YPD medium, and further cultured for 4 hours. After this, α-factor was added into the culture to induce cell-cycle synchronization and incubated for 2 hours. Following this, 0-min sample was collected, and remaining cells were washed, and released into fresh YPD at 28℃. Samples were collected by centrifuge every 10 minutes for 2 hours from the time of release.

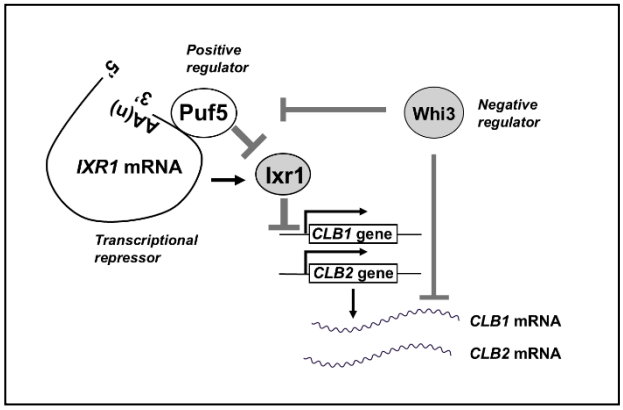
3. RNA Isolation and qRT-PCR

Total RNA extracted with ISOGEN (Nippon Gene), genomic DNA removed (RNeasy Mini, Qiagen), and cDNA synthesized (PrimeScript RT, Takara).qRT-PCR (QuantStudio 5, Thermo Fisher) with SYBR Premix Ex Taq (Takara) and the fold changes were normalized to *SCR1* or *ACT1* gene. Statistical analysis was done in Microsoft Excel.

■ KEY RESULTS:

1. Whi3 is a negative regulator of G2/M cyclins.
2. Whi3 indirectly regulates G2/M cyclins by influencing the Puf5-Ixr1 axis.
3. Whi3 supports genome stability and becomes essential under DNA damage and replication stress.

■ PROPOSED MODEL:



■ REFERENCES

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代表発表者 **Rana Varsha (ラナ ヴァーシャ)**
所　　属 **University of Tsukuba, Institute of Medicine, Laboratory of Molecular Cell Biology**
問合せ先 **〒305-8575 1-1-1 Tennoudai, Tsukuba, Ibaraki**
TEL & FAX:(+81) 29-853-3066
Email id: s2230542@u.tsukuba.ac.jp

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(3) Post-transcriptional

■共同研究者: Prof. Irie Kenji
University of Tsukuba, Institute of Medicine, Laboratory of Molecular Cell Biology