

# Revealing genetic interactions conferring robustness of the cell cycle in yeast by 2 dimensional genetic tug-of-war method.

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## Introduction

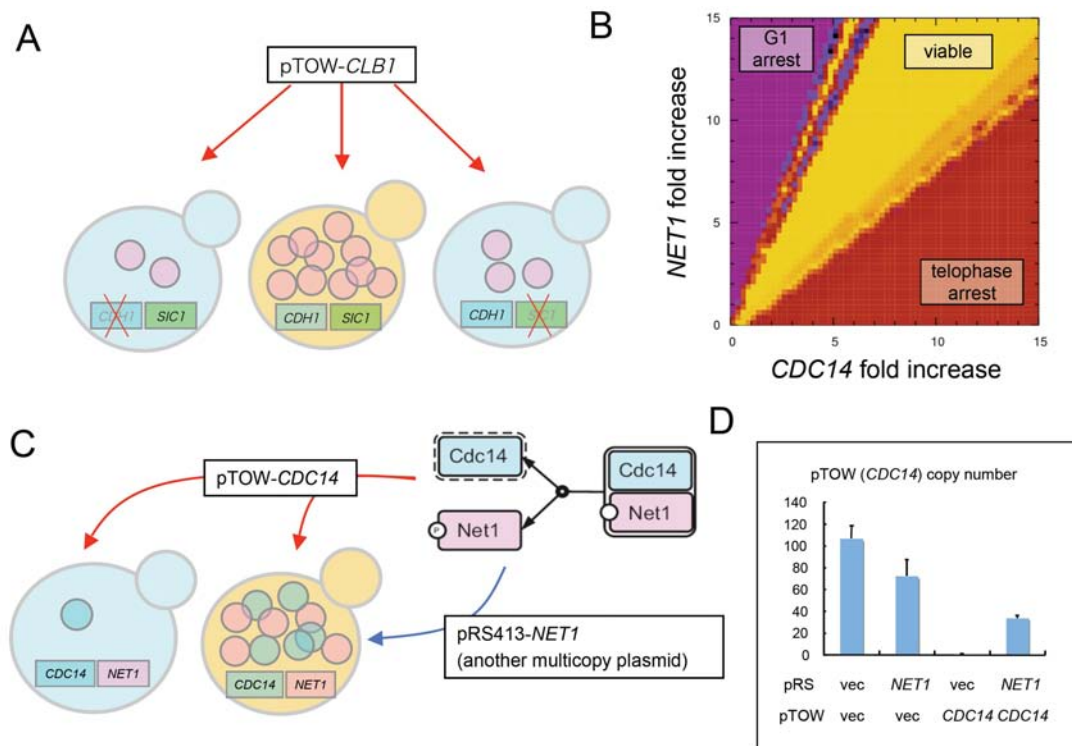
Robustness against perturbations, such as noise in gene expression or various mutations, is one of the fundamental properties of biological systems. Robustness of biological systems is considered to be developed through the long history of evolution. The robustness profiles of cellular subsystems are particularly informative in order to understand and control them, because they are emerged from the result of their complex molecular networks. Many approaches to understand cellular robustness have been performed with computer models with various kinds of methods, such as sensitivity analysis, bifurcation analysis etc.. However, little is known about the robustness (and fragility) of real cells due to difficulties in controlling intracellular parameters.

In previous report, we have developed a genetic method named 'genetic Tug-Of-War (gTOW)', which determines upper limit copy numbers of genes in the budding yeast *Saccharomyces cerevisiae*. This experiment was applied for the robustness analysis of 30 cell division cycle (CDC) related genes. The analysis revealed robustness profile of the yeast cell cycle (ref. 1). In this study, we have extended gTOW analysis in the yeast CDC system into 2-dimensional way by combination with knock-out mutations (KO), or another multicopy genes (double). We expect that 2D robustness profiling reveals molecular interactions conferring the robustness hidden in the complex network. Furthermore, the 2D data will be useful to validate computational models (such as ref. 2), and to improve them.

## Results and Discussion

**gTOW with KO:** A B-type cyclin gene *CLB1* has relatively higher upper limit gene dosage (about 100 copies per cell). To understand how this robustness is supported through the molecular network in the yeast CDC system, we quantified upper limits of *CLB1* in 19 *cdc* KO mutant cells. The upper limit of *CLB1* was significantly decreased by the deletion of *cdh1Δ* or *sic1Δ* (6.6, 5.9 fold reduction, respectively) (Fig. 1A). Cdh1 and Sic1 are known to function as opponents of B-type CDK, with redundant but different mechanisms. Our result suggests that although they are not essential elements in CDC system (*cdh1-sic1* double KO is lethal), their functional redundancy are conferring robustness against perturbation of B-type cyclin expression.

**gTOW-double:** A mitotic protein phosphatase *CDC14* has the lowest limit among 30 *CDC* genes (< 2.0 copy per cell). The sensitivity of *CDC14* is predicted by the computational model (ref. 2), and reasoned because of its stoichiometric regulation (i.e. regulated by protein-protein interaction) by an inhibitor Net1 (ref.1). The computer model further predicted that if *NET1* copy number is increased, *CDC14* can be increased as well (Fig 1B). We thus tested this prediction with gTOW combined with multicopy *NET1* plasmid (gTOW-double)(Fig. 1C). Same as the prediction, upper limit of *CDC14* was highly increased with the multicopy *NET1* (more than 30 fold, Fig. 1D). This finding strongly support the idea that the activity of Cdc14 is only regulated by the stoichiometric inhibition by Net1, and that is the reason why this subsystem is extremely fragile.



**Figure 1** (A) Principle of gTOW-KO, (B) computational prediction, (C) principle and (D) experimental result of gTOW-double are shown.

## Reference

1. Moriya H, Shimizu-Yoshida Y, Kitano H (2006) *In vivo* robustness analysis of cell division cycle genes in *Saccharomyces cerevisiae*. *PLoS Genet* 2(7): e111
2. Chen KC, Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ (2004) Integrative analysis of cell cycle control in budding yeast. *Mol Biol Cell* 15: 3841-3862

## Abbreviations

*CDC*: cell division cycle, gTOW: genetic Tug-Of-War, 2D: two dimensional, KO: knock-out, CDK: cycline-dependent kinase