Form 3

Abstract of Thesis

Name （Saeed Kaboli ）	
Title	Genome-wide mapping and phenotypic analysis of unexplored chromosomal regions responsible for synthetic lethality and synthetic haplo-insufficiency/proficiency in *Saccharomyces cerevisiae* (出芽酵母染色体領域の大規模削除による新規遺伝子間相互作用のゲノムワイドなマッピングと菌株育種への応用)

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One of the best strategies to integrate gene function into specific biological pathways at genome-wide level is large-scale mapping of genetic interaction networks. An important genetic interaction is synthetic lethal interaction where the combination of deletion of two genes is inviable while deletion of each gene is viable. Synthetic Genetic Array (SGA) method developed in *Saccharomyces cerevisiae* is a system in which a mutation in a specific query gene can be crossed to entire set of viable deletion mutants to map synthetic genetic interaction systematically. SGA has made it possible to systematize the efforts in detection of synthetic lethal interactions on a genomic scale. However, SGA is unable to evaluate combination of tightly linked gene-pairs because the construction of a double disruptant is not possible if the two genes to be disrupted are tightly linked on the same chromosome. Due to this problem, functional relationship, especially synthetic lethal interaction, between linked gene-pairs remains largely unknown.

From viewpoint of functional genomic analysis of diploid cell, despite previous work to discover genes that causes haplo-insufficiency (less fitness than parental diploid) or haplo-proficiency (high fitness than parental diploid) by using single knockout pools of the heterozygous collection, precise mapping of genetic interaction governing these phenotypes is not feasible in case of physically linked genes. Therefore, to elucidate responsible genetic network causing synthetic haplo-insufficiency or synthetic haplo-proficiency, construction of large chromosomal deletions in a diploid cell might be a useful approach. In this study, I investigated these ideas using *S. cerevisiae* haploid and diploid strain.

In this chapter, I attempted to delete 110 chromosomal regions found to harbor only non-essential genes as identified by single knockout experiments and longer than 10 kb by PCR-mediated chromosomal deletion technology (PCD), which enables chromosomal segments to be deleted by a one-step transformation. Results revealed that thirty-three of the 110 regions could be deleted, but the remaining 77 regions could not. To determine whether the 77 undeletable regions are essential, I converted 67 of them to mini-chromosomes marked with *URA3* using PCR-mediated chromosome splitting technology (PCS) which facilitates splitting a chromosome at any desired site. The essentiality of mini-chromosomes was tested by a mitotic loss assay of the mini-chromosomes using 5-FOA selection medium on which only Ura⁻ clone can grow. I found that 56 of the 67 regions were essential despite that they harbor only non-essential genes. I also found that 49 out of the 56 (87.5%) regions did not carry any of the co-lethal gene pair(s) previously identified. This implies that regions found to harbor only non-essential genes as identified by single knockout experiments in fact contain unidentified synthetic lethal combinations at unexpectedly high frequency.

Phenotypic analysis of 44 (33 identified by PCD and 11 identified by PCS) viable disruptants led to the following discoveries: Some strains exhibited a sensitive phenotype including a low-temperature and high-temperature sensitive phenotype; a ethanol-sensitive phenotype, and a sulfuric acid-sensitive phenotype. Interestingly, I also found that deletion of some regions confers more tolerant phenotype than the parent strain under high-temperature and lactic acid stress conditions. Taken together, these observations suggest that segmental chromosomal deletion in haploid might be exploited for not only revealing genome function but also breeding stress-tolerant strains in *S. cerevisiae*.

Deletion of one allele in homologous chromosomes in diploid organism occasionally causes phenotypic alteration. *S. cerevisiae* strains collected from different kinds of industrial and natural geographical environments were reported to have great variation in copy number of telomere and sub-telomere regions (Dunn et al., 2012, Stambuk et al., 2009). This suggest that segmental haploidization of subtelomere regions in diploid may confer adaptive force in fermentation-related stress environments. However, an earnest analysis with this prediction is lacking. Therefore, I tested this idea by constructing diploid with segmental deletion (called segmentally haploidized strain) of every 200 kb regions covering all of 32 terminal regions of one of homologous chromosomes by PCD method. Two among 32 terminal regions could not be deleted by PCD method, suggesting that such karyotype may be lethal. Some of remaining 30 segmentally haploidized strains were sensitive to stressful conditions tested. Interestingly, four strains were stress-tolerant to lactic acid (5%; pH=2.7) and high temperature (41°C). Four segmentally haploidized strains exhibited significant improvement of ethanol production (3%, 3%, 2.9% and 2%, respectively) compared to the wild-type diploid strain. Then, I investigated the effect of combinatorial haploidization by two regions each of whose deletion causes improved productivity of ethanol. Results revealed that strain having simultaneous deletion of two regions displayed 4.7% higher ethanol productivity compared to the wild-type strain, indicating that combinatorial deletion of some chromosomal regions leads to further improvement of industrially important phenotype.

This work describes how segmental chromosomal deletion in haploid and diploid could be exploited in both revealing genome function and breeding of *S. cerevisiae*. All of the essential regions discovered for the first time in this study likely contain lethal interactions of linked gene-pairs, indicating that combined strategy of PCD and PCS for detecting these genes would have a significant impact on revealing novel landscape of genetic interaction in *S. cerevisiae* genome. Although phenotypic consequences of segmental deletion might generally be detrimental irrespective of haploid or diploid, our result shows that this type of deletion occasionally confers a growth advantage and stress-tolerant phenotypes. Taking these facts into account, segmental deletion of chromosome is an unique approach providing additional insights into understanding genetic network and strain breeding of *S. cerevisiae*.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

One of the best strategies to integrate gene function into specific biological pathways at genome-wide level is large-scale mapping of genetic interaction networks. An important genetic interaction is synthetic lethal interaction where the combination of deletion of two genes is inviable while deletion of each gene is viable. Synthetic Genetic Array (SGA) method developed in *Saccharomyces cerevisiae* is a system in which a mutation in a specific query gene can be crossed to entire set of viable deletion mutants to map synthetic genetic interaction systematically. SGA has made it possible to systematize the efforts in detection of synthetic lethal interactions on a genomic scale. However, SGA is unable to evaluate combination of tightly linked gene-pairs because the construction of a double disruptant is not possible if the two genes to be disrupted are tightly linked on the same chromosome. Due to this problem, functional relationship, especially synthetic lethal interaction, between linked gene-pairs remains largely unknown. From viewpoint of functional genomic analysis of diploid cell, despite previous work to discover genes that causes haplo-insufficiency (less fitness than parental diploid) or haplo-proficiency (high fitness than parental diploid) by using single knockout pools of the heterozygous collection, precise mapping of genetic interaction governing these phenotypes is not feasible in case of physically linked genes. Therefore, to elucidate responsible genetic network causing synthetic haplo-insufficiency or synthetic haplo-proficiency, construction of large chromosomal deletions in a diploid cell might be a useful approach. In this study, I investigated these ideas using *S. cerevisiae* haploid and diploid strain.

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