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学位論文題目　Identification and characterization of genes which are regulated by Ras GTPase-mediated signal transduction pathway in *Schizosaccharomyces pombe*.

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論文内容の要旨

The Ras GTPase plays a central role in the signal transduction from the cell exterior to the nucleus. These signals elicit very specific cellular responses such as cell proliferation and cell differentiation depending on the stimuli. Fission yeast ras1 plays an important role in two distinctive signal transduction pathways, mating pathway and cytoskeleton organization pathway. The former process occurs upon nutritional stress usually when cells are starved of nitrogen. Cells of two opposite mating types, h^+ and h^-, conjugate to produce a diploid zygote, which in turn undergoes meiosis and sporulation, yielding an ascus containing four haploid spores. The *ras1* mutant strains are incapable of conjugation and are highly inefficient in sporulation.

Two protein kinases, Byr1 and Byr2 are known to be capable of the partial phenotypic suppression of *ras1* mutation. It is known that Ras1 mediates sexual differentiation through the well-conserved MAP kinase pathway composed of Byr2, Byr1, and Spk1 kinase upon nutrient starvation. In addition to the sexual differentiation, Ras1 is involved in cytoskeleton organization. *ras1* mutant strains have abnormally round cell morphology compared to rod-shaped morphology of wild type cells. To regulate the organization of the cytoskeleton, Ras1 interact with Scd1, which is a putative GEF for Cdc42sp, Cdc42sp in turn regulates Shk which is a member of the conserved p21-activated protein kinase family. In spite of extensive studies on the Ras1-mediated signal transduction pathway, there was no convincing report on factors that could recover the Ras1 function entirely when expressed in *ras1* mutant strains. This suggests that Ras1 may be involved in multiple signal transduction pathways that are not revealed yet.

To clarify the ras1 function in fission yeast, it is necessary to understand overallview of genes regulated by Ras1 mediated signaling pathway; to know the ras1 function clearly in fission yeast, the DNA microarray containing 13,824 *S. pombe* genomic clones was prepared. Using this DNA chip, the author screened differentially-expressed genes between *ras1* mutant and wild type cells. Total of 305 clones was identified to show over 2-fold increase or decrease. Among them, the author selected 38 clones that showed 2.5-fold of increase or decrease. The author analyzed several characteristics of these clones to know function related to their expression.

First, in *ras1* mutant cells, the factors controlling ion transport were increased compared to that of wild type cells (ferric reductase, calcium permease, and sodium ion / proton antiporter). This result may imply that factors related to the ion transport or homeostasis are under the control of RasGTPase signaling pathway. Second, the factors related to the stress response were also increased in ras1 mutant cells (putative sensory transduction histidine kinase and longevity assurance factor).

Third, transcription factor whose function is unknown was also increased in *ras1* mutant cells.

It was reported that the transcription factor AFT1 controlling gene expression of ferric reductase was involved in cell size control in *S. cerevisiae*. For this reason, the author focused his attention on gene coding for ferric reductase that was increased in *ras1* mutant cells. Through the northern blot analysis, the author confirmed that the expression of genes coding for ferric reductase was increased in ras1 mutant cell. A ferric reductase in *S. cerevisiae*, is involved in uptake of iron and copper ions.

The author thought that ras1 mutant cells would be resistant against starvation of iron and copper ion and this fact was verified through the starvation experiment of iron and copper ion. This result suggests that *ras1* mutant cells are resistant against to iron and copper ion starvation compared to that of wild type cell. Increased expression of ferric reductase is related to the resistance to iron and copper starvation. In fission yeast, the mutant for ion homeostasis has the defect in cell wall integrity. It was revealed that Ras1 mutant cells are sensitive to treatment of cell wall lysis enzyme through the β-glucanase sensitivity experiment. This result suggests that cell wall composition of ras1 mutant cell is changed compared to that of wild cells.

Taken together, this study suggests that Ras1 might involved in signaling pathway controlling cell wall integrity and ion homeostasis as well as mating and cytoskeleton in fission yeast. In this study, the author described the identification and characterization of genes that are regulated by RasGTPase in fission yeast.

論文の審査結果の要旨

本学位申請論文『Identification and characterization of genes which are regulated by RasGTPase-mediated signal transduction pathway in Schizosaccharomyces pombe』は、ras遺伝子の制御下にある遺伝子群を網羅的に解析することを目的としている。Ras 蛋白質は動物細胞の様々なシグナル伝達系において、分子スイッチとして細胞の増殖と分化に重要な役割を果たしている。分裂酵母においては、一個のras遺伝子ras 1 が知られており、細胞の形態維持と、栄養源枯渇下での接合および胞子形成過程において、細胞内情報伝達系で重要な役割を果たしている。分裂酵母の野生型株の細胞形態は円筒形であるのに対して、ras 1 変異株の形態は太く短い。栄養源が枯渇すると、一倍体同士での接合および二倍体株の減数分裂と胞子形成を行うが、ras1変異株の一倍体は接合不能になり、ras1を欠く二倍体では胞子形成率に著しい低下が見られる。本学位論文の研究においては、分裂酵母ras 1 の遺伝子破壊株を作成し、野生株と遺伝子破壊株での遺伝子発現の差違を、DNAマイクロアレイを用いて定量解析している。具体的には、*S. pombe* の数kb程度のgenomic DNAをクローン化した約14,000種のplasmid DNAをプリントしたDNA chipを用い、ras 1 の遺伝子破壊株と野生株の各々から調製したRNAを異なった蛍光色素で標識したfirst strand cDNAをプローブとして、マイクロアレイ解析を行った。2.5倍以上の遺伝子発現の差が認められた約40のクローン化DNAについて、塩基配列の決定を行い、ras 1 の遺伝子破壊の影響を受ける遺伝子群を特定している。結果として、イオンの透過性および恒常性に関係ある遺伝子の発現がras 1 破壊株で増加することを明らかにした。この遺伝子破壊株で鉄と銅イオンの欠乏に対して顕著な抵抗性を示すことを確認している。さらに、ras 1 遺伝子破壊株は細胞壁の変化により、β-glucanase処理に感受性を増すとの知見も得ている。これらの結果を総合して、分裂酵母において、Rasは細胞壁の integrityとイオン恒常性を調節する情報伝達系に深く関係することを結論している。本学位論文の研究により、分裂酵母のras 1 が制御する遺伝子群に関する基礎的な知見が得られただけでなく、分裂酵母の遺伝子発現の全体像を解析する実験システムが確立したことの意義は大きい。本論文は、学位論文としてふさわしい内容を持っていると認められる。