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学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第４条第１項該当
学　位　論　文　題　目	細胞周期進行を制御する新しい分裂酵母ユビキチン結合酵素(ubcP4)
論文審査委員	主　査　教　授　廣　瀬　　進 教　授　石　浜　　明 助教授　小　原　雄　治 助教授　藤　山　秋左夫 教　授　廣　瀬　　進（遺伝学専攻）

論文内容の要旨

The ubiquitin system is one component of the biological regulatory mechanisms, and is related in a variety of cellular processes including the cell cycle control, DNA repair, stress response and transcriptional control. Ubiquitin, a highly conserved eukaryotic protein, can be found either free or bond to the various substrate proteins. The covalent attachment of ubiquitin to the substrate proteins is accomplished by the sequential reaction of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3). The ubiquitin-substrate conjugates are rapidly degraded by the 26S proteasome, a multisubunit complex of protease.

To date, a number of different E2s have been characterized. All known E2s share a conserved domain of approximately 16kDa and a specific cystein residue which is capable of accepting the ubiquitin. It is thought that the diversity of E2s is required for the selective ubiquitination of the substrate proteins which mediate different cellular functions.

To isolate E2s systematically, he has developed a new screening method which utilized a common activity of E2 enzymes. Consequently, he has been isolated four members of a family of E2s from the fission yeast *Schizosaccharomyces pombe*, and termed them *ubcP1 - 4*. Comparison of the deduced amino acid sequences of *ubcP1 - 4* cDNA with all of so far known E2s from the various organisms revealed that *ubcP4* is a novel member of a family of E2s. The deduced amino acid sequence of *ubcP4* is 20 - 30% identical to a family of E2s and contains a specific cystein residue which is capable of accepting the ubiquitin.

Disruption of the coding region of *ubcP4* gene shows that *ubcP4* is an essential gene for growth in the fission yeast. The repression of the *UbcP4* synthesis caused the several phenotypes such as G2 arrest, metaphase arrest and cut (cell untimely torn). The detailed phenotype analysis revealed that *ubcP4* is required for the both cell cycle transitions; from G2 to M phase and from metaphase to anaphase. Interestingly, the several phenotypes in metaphase/anaphase caused by the loss of *ubcP4* function, are strikingly similar to those of *cut9* ts mutants; *cut9* is also required for the metaphase/anaphase transition and is a potential component of ubiquitin ligase (E3), termed anaphase-promoting complex (APC) or cyclosome. Therefore, he concludes from the phenotype similarities that *ubcP4* may function in cooperation with APC at metaphase/anaphase.

"Checkpoint controls" delay mitosis until DNA synthesis and repair of DNA damage are complete. The DNA damage-induced delay is mainly due to a temporary G2 arrest and provide the time necessary for the cell to complete DNA

repair before mitosis. In this study, he showed that the amount of *UbcP4* is decreased in response to ultraviolet irradiation, and the cells overexpressing *ubcP4* are sensitive to ultraviolet irradiation. These data suggest that the decreased level of *UbcP4* is required for the cells when DNA is damaged. Since the repression of *ubcP4* synthesis caused the G2 arrest, he concludes that the specific ubiquitin-pathway involving *ubcP4* could be required for the DNA damage-induced G2 arrest.

In conclusion, he has isolated a novel member of a family of E2s, *ubcP4*, and showed that it is required for the both of G2/M transition and metaphase/anaphase transition, and possibly for the checkpoint control in response to DNA damage.

論文の審査結果の要旨

ユビキチン系の構成因子であるユビキチン結合酵素（E2）は分子ファミリーを形成しており、その分子多様性が種々の基質蛋白質を選抜的に認識することを可能にしている。本研究で逢坂文男君は、E2 が持つ共通の酵素活性を指標とした新しいスクリーニング系を開発し、分裂酵素から４種類のE2、*ubcP1～4* をクローニングした。

ubcP1～3 は既に出芽酵母からクローニングされている E2 のホモログであったが、*ubcP4* は現在までに報告されている全ての E2 分子種とは高い相同性がなく、新しい分子種であることが判明した。*ubcP4* は必須遺伝子であり、*ubcP4* 欠損株ではＧ２期からＭ期への移行と、metaphase から anaphase への移行の両方が阻害されていた。metaphase で停止した細胞の表現型は、ユビキチン系の E3 の成分であることが示唆されている*cut9* 遺伝子の温度感受性変異株の表現型と酷似しており、*cut9* と *ubcP4* が同じユビキチン経路で働いていることが推測された。一方、Ｇ２期での停止に関しては、*ubcP4* が DNA 損傷時のチェックポイントコントロールで機能することが示唆された。

以上のように、この論文の内容はE2 の研究に新しい方向性を与える可能性を秘めており、遺伝学専攻の博士論文としての条件を満たすものであることを、審査委員全員が認めた。