108 年度生醫系專題研究競賽報名表

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題目(中文):利用四環素調節的雙分子螢光互補方法驗證 Jhd2 和 Not4 蛋白質在白色念珠菌具交互作用

(英文):The use of a tetracycline-regulated bimolecular fluorescence complementation (BiFC) assay to verify the interaction between Jhd2 and Not4 in *Candida albicans*

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- 摘要:

Candida albicans is a member of commensal flora in healthy human body. However, it serves as opportunistic fungal pathogen in the immunocompromised patients, such as HIV infection and chemotherapy. Recently, *C. albicans* was reported as the 4th pathogen of healthcare-acquired infections in the ICU of Taiwan. If the care of infection was out of control, invasive candidiasis would severely lead to death. Morphological plasticity of *C. albicans* adapted to variety of environment contributed to its pathogenesis.

Gene expression depended on the methylation of histone H3 at Lys4 has been known in eukaryotes. In *Saccharomyces cerevisiae*, JmjC domain-containing H3K4 demethylase (Jhd2) solely antagonizes the trimethylation of H3K4 state, and is broken down through ubiquitination catalyzed by Not4, which is an ubiquitin ligase in the CCR4-NOT complex. However, the interaction between Jhd2 and Not4 associated with morphogenesis in *C. albicans* is unclear.

In this study, tetracycline-regulated bimolecular fluorescence complementation assay (BiFC) was utilized to validate the interaction between *Ca*Not4 and *Ca*Jhd2 *in vivo*. Split-mCherry complex is used as the reporter. We constructed the Tet-on systems capable of expressing either RN (1-159 amino acid of mCherry) or RC (160-237 amino acid of mCherry) fused to *Ca*Not4 or *Ca*Jhd2. These two Tet-on systems carrying a hygromycin B selectable marker (*CaHygB*) and a nourseothricin selectable marker (*CaSAT1*), respectively, were transformed into the *C. albicans* strains RBY717 (a/a) and RBY722 (α/α) which possess mating competency after transformed into opaque state. By mating of opaque cells with Tet-on systems, the BiFC vectors will be efficiently implemented into a *C. albicans* cell. Red fluorescence observed under a fluorescent microscope is expected when the interactions occur.

At the same time, the timing of *Ca*Not4-*Ca*Jhd2 interaction will be addressed through a variety of morphological stimuli, including serum at 37°C, nutrient starvation, cell cycle arrest or acidic and basic pH. After verifying the interaction between *Ca*Jhd2 and *Ca*Not4, I will perform immunoprecipitation of *Ca*Jhd2 followed by a western blotting to verify the presence of ubiquitinated Jhd2. In addition, the location of the interaction should be predicted in the nucleus and the intensity of red fluorescence will be quantified under different conditions.

In conclusion, the BiFC system allows effective visual assay for *Ca*Not4 and *Ca*Jhd2 interactions in a *C. albicans* cell. Combined with chemical genetics and biochemical assay, it helps us understanding the relationship between *Ca*Not4 and *Ca*Jhd2 in the morphogenesis of *C. abicans*.

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