We isolated Tn10-insertion mutants affecting the activity of SoxR, screening for constitutive expression of SoxS using *soxS-lacZ* fusion. One of the mutations was mapped in *rseB*, a gene in *rseABC* (Regulation of SigmaE) operon. The constitutive *soxS*-expressing phenotype was due to the polar mutation of downstream gene, *rseC*. RseC is likely to function as a component of SoxR reduction system because SoxR was kept in oxidized form to activate *soxS* expression in *rseC* mutant. RseC is a membrane protein with a cysteine-rich N-terminal domain facing the cytoplasm and a transmembrane domain in the C-terminal region. The functionally critical cysteines were determined by cysteine to serine substitution mutagenesis. The transmembrane domain of RseC was also required for RseC function in reducing SoxR. The truncated N-terminal domain of RseC reduced the *soxS* transcription by 50% as judged by in vitro transcription assay. RseC was subject to conformational change according to the redox condition and had an antioxidant activity in vitro. RseC-overproducing cell became more resistant to H_2O_2 and cumene hydroperoxide in vivo but, not to the superoxide generating agents, such as menadione and plumbagin. The phenotype of *rseC* mutant in the stationary phase revealed some interesting properties. Expression of hydroperoxidase I (KatG) did not increase and the expression level of SoxS remained high, although SoxRS system is silent to superoxide generating agents in the stationary phase.

SL304

Regulation of Pseudohyphal Growth in *Candida albicans*

Cheol-Sang Hwang* and Sa-Ouk Kang
Laboratory of Biophysics, School of Biological Sciences, Graduate School, and Institutes of Microbiology, Seoul National University, Seoul 151-742

The pathogenic fungus, *Candida albicans*, undergoes reversible morphogenetic transition ranging among budding yeast, true hypha and pseudohypha. Although pseudohyphae, which were considered as the third form, vary in shape from attached strings of yeast-like cells to long filaments with constriction at the septa, the developmental process has not yet established. A *C. albicans PRF1* (pseudohypha- regulating factor) gene that encoded a protein highly homologous to *Saccharomyces cerevisiae* Ssn6p and *Dictyostelium discoideum* TRFA was isolated. Mutants lacking Prf1p did not develop into true hypha, but rather grew exclusively as pseudohyphae on a variety of aerobic conditions tested at hyphal inducing temperature (37 °C). Under anaerobic or embedded condition, the *prf1/prf1* cells showed a markedly suppressed filamentous growth. Furthermore, the *prf1/prf1* strains exhibited a severe growth defect in serum at 37°C and were unable to established systemic infection in mice. Thus Prf1p is an important regulator determining the morphological transition and virulence in *C. albicans*, and may be a putative target for the exploration of candidacidal drug.

SL305

Identification of a Domain in Yeast Chitin Synthase 3 Required for Biogenesis of Chitin Ring, But Not Cellular Chitin Synthesis

Hyun-Sook Park, Mee-Hyun Park, Chi-Hwa Kim, Jeeun Woo,
Jee-Yeon Lee, Sung-Uk Kim[1] and Wonja Choi
Department of Biological Sciences, Ewha Womans University, Seoul 120-750; [1]Korea Research Institute of Bioscience and Biotechnology, P.O.Box 115, Yusung, Daejon 305-333

Chitin, the most abundant polymer

next to cellulose, is found in a majoity of fungal cell wall and septa. In *Saccharomyces cerevisiae,* chitin constitutes small portion of cell wall, but is indispensible for cell viability (Shaw *et al.,* 1991). Its synthesis is catalyzed by chitin synthases which are found as multiple isozymes in many fungi (Bowen *et al.,* 1992). Three chitin synthase genes (*CHS1, CHS2* and *CHS3*) have been described in *S. cerevisiae* (Bulawa *et al.* 1986; Sburlati and Cabib 1986; Silverman *et al.,* 1988; Valdivieso *et al.,* 1991). They share high structural homology and carry out same biochemical reactions but play distinctive roles throughout the cell cycle. Thus the activity of each enzyme should be tightly regulated to exert its functions not only stage-specifically but also site-specifically.

It has been proposed that *CHS3*-mediated chitin synthesis during the vegitative cell cycle is regulated by *CHS4.* To investigate direct protein-protein interaction between their coding products, we used yeast two hybrid system and found that a domain of Chs3p was responsible for interaction with Chs4p. This domain, termed MIRC3-4 (maximum interacting region of chs3p with chs4p), spans from 647 to 700 residues It is well conserved among *CHS3* homologs of various fungi such as *Candida albicans, Emericella nidulans, Neurospora crassa, Magnaporthe grisea, Ustilago maydis, Glomus versiforme, Exophiala dermatitidis, Rhizopus microsporus.* A series of mutaion in the MIRC3-4 resulted in no appearance of chitin ring at the early G1 phase but did not affect chitin synthesis in the cell wall after cytokinesis. Absence of chitin ring could be caused either by delocalization of Chs3p to the septum or by improper interaction with Chs4p. To discriminate those two, not mutually exclusive, alternatives, mutants cells were immunostained with Chs3p-specific antibody. Some exhibited localization of Chs3p to the septum, while others failed. These results indicate that simultaneous localization and activation Chs3p by Chs4p is required for chitin ring synthesis. *CHS4* played its dual roles by expressing in G1 and cytokinesis which corresponded with CSIII activity during the cell cycle.

SL306

Bypassing Immunization: Optimized Design of Designer T Cells against CEA Expressing Tumors

Chae-Ok Yun, Kath F. Nolan[1], Yoshiko Akamatsu[1] and Richard P. Junghans[1]

Yonsei Cancer Center, Institute for Cancer Research, College of Medicine, Yonsei University, Seoul 120-749; [1]Biotherapeutics Development Lab, Harvard Institute of Human Genetics, Harvard Medical School, and Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Boston, MA 02215, U.S.A.

Tumor-associated antigens are typically nonimmunogenic in cancer patients, "immune surveillance" having manifestly failed. The fact that most tumor antigens are normal human proteins presents significant obstacles to current cancer immunization approaches that researchers are presently striving to overcom. An alternative strategy bypasses immunization altogether by direct genetic alteration of autologous patient T cells, to create "designer T cells" specific to a particular antigen. Chimeric immunoglobulin-T cell receptors (IgTCR) with a specificity for carcinoembryonic antigen (CEA) were created to evaluate the optimal IgTCR structure for cancer therapy. Antigen-binding domains of a humanized antibody were combined with TCR signaling chains to yield four different chimeric IgTCRL single chain Fv fragment (sFv)-, fragment antigen-binding (Fab)-, sFv-, and Fab-. All of the IgTCR were well expressed on T cells, and all showed specific binding and activation, as