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.

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

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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

demonstrated by IL-2 production on contact with immobilized or cellular CEA. In contrast to prior studies of isolated TCR chains that related increased tyrosine-based activation motifs in as a reason for superior signaling potency, these tests are the first to show that and are indistinguishable for T cell signaling when assayed in the context of the intact TCR complex. Further, Fab was equivalent to sFv as an IgTCR component for expression and antigen binding. When IgTCR was expressed on normal human T cells, cytotoxic potency was demonstrated at low E:T ratios, with T cell recycling and progressive tumor cell destruction. These studies establish a potentially important new immnotherapeutic modality for the treatment of CEA-expressing tumors.

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JAK Kinases and STAT Proteins in IL-12 Receptor Signaling

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IL-12 activate JAK2 and TYK2 and induce the phosphorylation of STAT4 and STAT3, but little is known how the activation of these signaling molecules is related to the biologic effects of IL-12. Using an IL-12-responsive T cell clone, We investigated their requirements for proliferation and IFN-g production of 2D6 cells. 2D6 lines maintained with IL-12 (2D6-12) or IL-2 (2D6-2) exhibited comparable levels of proliferation, but produced large or only small amounts of IFN-g, respectively, when restimulated with IL-12 after starvation of either cytokine. 2D6-12 induced TYK2 and STAT4 phosphorylation. But their phosphorylation was dramatically reduced in 2D6-2. The reduced STAT4 phosphorylation was due to a progressive decrease in the amount of STAT4 protein along with the passages in IL-2-containing medium. 2D6-12 and 2D6-2 similarly proliferating in response to IL-12 induced comparable levels of JAK2 activation and STAT5 phosphorylation. JAK2 was associated with STAT5, and IL-12-induced STAT5 phosphorylation was elicited in the absence of JAK3 activation. Above results also were confirmed in the Con A blast of B6 mouse spleen. These results indicate that TYK2 and JAK2 activation correlate with STAT4 phosphorylation/IFN-g induction and STAT5 phosphorylation/ cellular proliferation, respectively.

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Superinfection Exclusion of BVDV Occurs Not Only at the Level of Structural Protein-dispensable Viral Replication But Also at the Level of Structural Protein-required Viral Entry

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For a variety of viruses, the primary virus infection has been shown to prevent superinfection with a homologous secondary virus; however, the mechanism of exclusion has not been clearly understood. In this work, we demonstrated that BVDV-infected MDBK cells were protected from superinfection with a homologous superinfecting BVDV, one of the positive-sense RNA pestiviruses, but not with an unrelated rhabdovirus, such as vesicular stomatitis virus. Once superinfection exclusion was established by a primary infection with BVDV, the transfected infectious BVD viral RNA genome was shown to be competent for viral translation, but not viral replication. In addition, our results