

**P018** Isolation of mutants defective in sterol transport in yeast  
**David P. Sullivan** and **Anant K. Menon**  
*Department of Biochemistry, Weill Medical College of Cornell  
University, New York, USA*

We recently showed that transport of ergosterol from the endoplasmic reticulum (ER) to the sterol-enriched plasma membrane (PM) is unaltered in yeast cells defective in Sec18p, a protein required for most intracellular vesicular traffic. Thus ER-PM sterol transport likely occurs by a non-vesicular (Sec18p-independent) mechanism. To probe these possibilities we identified novel yeast mutants defective in ER-PM sterol transport. We used a strain of yeast that takes up sterols under aerobic conditions – when cholesterol is added to these cells it is transported to the ER, esterified and sequestered in lipid droplets. Defects in any aspect of the uptake-transport-esterification pathway are expected to decrease the level of accumulated sterol ester. We used this pathway as a basis for tritium suicide selection of mutants defective in sterol transport. After chemical mutagenesis we loaded the cells with [<sup>3</sup>H]cholesterol and placed them at -80°C. Cells with high accumulation of [<sup>3</sup>H]cholesterol are expected to die as a result of radiation damage whereas mutants in the uptake pathway are expected to survive. We recovered a number of mutants defective in sterol transport between the ER and PM. Further characterization of the mutants will be presented.