Tuesday. Symposium III: Plant Cell Biology (abstracts not available)

Tuesday. Symposium IV: Yeast Cell Biology (544)

A Cycle of GTP Hydrolysis Regulates Vesicle Budding in the Secretary Pathway. R. Schekman, T. Yoshida, G. Sarnow, A. Emr, Y. Moriya, and H. Rapoport. Department of Molecular and Cell Biology, Howard Hughes Medical Institute, Berkeley, CA. A set of at least six genes (SEC6, SEC8, SEC16, SEC23, SEC24, SECl2) are required for transport vesicle budding from the endoplasmic reticulum in S. cerevisiae. These genes have been reproduced with isolated ER membranes and pure cytosolic and peripheral membrane Sec proteins. The budding reaction is monitored by the transfer of a radioactive secretory protein, yeast α-factor precursor, from rapidly-sedimenting ER membranes to slowly-sedimenting transport vesicles. Starting with crude and salt-washed membranes, budding requires three soluble protein fractions (Sec6p, Sec12p complex, and Sec23/24p complex), and hydrolysable ATP and GTP. An integral membrane protein, Sec12p, contains a N-terminal, cytosolically-exposed domain that is also required for budding. Deletion of the membrane anchor of Sec12p and C-terminal luminal domain of Sec12p generates a soluble N-terminal fragment that is competent for budding. The target of this inhibitory effect is Sarl; addition of excess Sarl overcomes inhibition. Sarl, Sec12p, and Sec33p are linked in a cycle of GTP hydrolysis and nucleotide exchange that require detergent or phospholipid. GTP hydrolysis by Sarl is stimulated tenfold by the Sec33p subunit of the 23/24p complex. GTP-GDP nucleotide exchange on Sarl is stimulated fivefold by the cytosolic domain of Sec12p. The signal that triggers nucleotide exchange, the target of GTP-Sarlp, and the mechanism coupling to vesicle budding are open questions.

Minisymposium 7: Extracellular Matrix and Cell Signaling (545–546)

Integrin-dependent Regulation of Tyrosine Phosphorylation in Platelets

J.S. Brugge 1, L. Lifften 1, B. Haimovich 1, E.A. Clark 1, J. D. Schaller 2, B.S. Cobb 3, J.T. Parsons 4, J.E. Fox 5, and P. J. Shaw 1. Lewis Research Institute, Cambridge, MA. We have used platelets as a model system to investigate the mechanisms involved in signal transduction mediated by the integrin family of adhesion molecules. The induction of tyrosine phosphorylation of multiple platelet proteins has been shown to be dependent on platelet aggregation mediated by fibrinogen binding to the integrin, GPIbα-IIIa. In addition, spreading of platelets on a fibrinogen matrix. The association of several platelet proteins has been recently identified as a protein tyrosine kinase, piP25FAM, as one of the proteins that is phosphorylated on tyrosine in platelets activated with thrombin or spread on a fibrinogen matrix. GPIbα-dependent tyrosine phosphorylation is dependent on platelet aggregation mediated by GPIbα-IIIa in thrombin treated platelets. In addition, we have found that piP25FAM is a major protein from activated platelet display, activated levels of phosphorylated in immune-complex assays, suggesting that the catalytic activity of piP25FAM is activated. Furthermore, we have shown that the protein tyrosine kinase, pTyr-PK, associates with the 15kDa Triton-insoluble cytoskeletal fraction following thrombin treatment, and that this redistribution of Src is also GPIbα-IIIa-dependent. Several lines of evidence indicate that upon activation of platelets, Src molecules are activated prior to platelet aggregation, and then redistribute to the Triton-insoluble cell fraction, suggesting that GPIbα-IIIa-dependent cytoskeletal protein complexes may serve as anchors for Src. We have found that Fyn, Lyn, and Yes also redistribute to the cytoskeleton in a GPIbα-IIIa-dependent fashion. Together these results suggest that integrin-cytoskeletal protein complexes may serve to compartmentalize and anchor activated enzymes involved in signal transduction.