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Regulation of Protein Transport in Eukaryotic Cells

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Eukaryotic cells are subdivided into a variety of compartments, each with its own complement of proteins. Targeted transportation of proteins is required not only to establish this complex network of organelles, but also to allow newly synthesized proteins to be secreted. Protein transport in the secretory and endocytic pathway is mediated by small vesicles that bud from one compartment and deliver their cargo after fusing with a defined acceptor compartment. Transport vesicle formation and fusion are cellular activities that require complex molecular machines whose multiple components are evolutionarily highly conserved. Specificity and directionality of vesicular transport, therefore, relies on the same functional principles regardless of whether single-celled organisms, like yeasts, or human brain cells are concerned. Genetic studies with yeast and biochemical investigations with cell- free transport systems from yeast and mammalian cells have contributed most to our present knowledge in this field of cell biology. Different aspects of protein transport through the secretory and endocytic routes in yeast will be discussed, and emphasis will be on the regulatory role of small GTPases of the Ypt/Rab family.

Ypt/Rab-GTPases are thought to recruit various effector proteins to the target membrane to which a given vesicle population docks and with which it fuses. Tethering of ER-derived vesicles to Golgi membranes requires Ypt1p in yeast (Rab1 in mammalian cells). As GTPases are specific for different transport steps, it is likely that each of the eleven yeast Ypt-GTPases is recognized at the relevant acceptor membrane by a specific receptor. A complex of two integral membrane proteins possibly fulfilling the role of such a receptor has been identified (1, 2). Like Ras proteins, Ypt/Rab-GTPases are active in their GTP-bound state, and for down-regulation, GTPase-activating proteins (GAPs) are needed to accelerate the very slow intrinsic GTPase activity of the regulators. By a combination of genetic and biochemical methods, we previously identified the first Ypt/Rab-specific GAP (3). Further studies showed that Ypt/Rab-GAPs in eukaryotic cells contain several conserved sequence elements, one harbouring an arginine residue essential for the 10^5-10^6fold acceleration of the GTPases' intrinsic GTP hydrolytic activity
(4) . The critical arginine in Ypt/Rab-GAPs most likely acts, like an arginine in Ras-GAP, as a so- called "arginine finger". This is backed by the recent elucidation of the X-ray crystal structure of the catalytic domain of a Ypt1-GAP, Gyp1p
(5) . In yeast, there are three Ypt1-GAPs, Gyp1p, Gyp5p and Gyp8p. Their inactivation following deletion of all three genes leads to defects in cell growth and to inappropriate vesicle fusion
(6) . We will also report on the functioning of the GTPase Ypt6p that presumably acts at several steps of retrograde vesicle transport from endosome(s) to the ER (7).

Aside from Ypt/Rab-GTPases, fusion of vesicle and acceptor organelle membranes requires several other stage-specific proteins, among them membrane receptors, SNAREs, and SNARE-binding proteins of the Sec1 family, also termed SM proteins. Sec1 family members play a positive role in membrane fusion. Their 30- structure is conserved (8). Sly1p, the SM protein essential for ER-to-Golgi transport in yeast, binds the Golgi SNARE Sed5p with high affinity and appears to contribute to the specificity of SNARE fusion complex formation (9).

The few examples of structure and function of components participating in vesicular protein transport which are described here can only give a glimpse of the complexity of this essential cellular activity. Consequently, this abstract only cites a few papers of our own work.

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