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Analysis of Kinectin and Small GTPases on Peroxisomes

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Previous studies on peroxisomal motility in CHO cells indicated the involvement of RhoA and kinectin in regulating the intracellular movement of peroxisomes. Specific affectors of RhoA, such as *C. botulinum* exoenzyme C3, increased peroxisomal movements by facilitating the cytoskeletal-organellar association. Since such associations may be mediated by organelle-linker proteins, such as kinectin, the existence of a peroxisomal kinectin was investigated. Both biochemical and immunological studies provided evidence that a distinct kinectin isoform is localized to peroxisomes. Interestingly, kinectin contains a RhoA binding site and thus may represent the site of regulation of peroxisomal movements by RhoA. Using specific antibodies directed against RhoA, the GTP-dependent recruitment of the protein from the cytosol to peroxisomes was established. The findings of RhoA interaction with peroxisomes raised the possibility that other small GTPases may still act on peroxisomes. Therefore, 2D electrophoresis in combination with a GTP-overlay technique was performed in order to visualize these GTPases. Extensive investigations were carried out in order to optimize the 2D electrophoretic procedure for separating and identifying peroxisomal membrane proteins. Finally, these studies provided evidence for the peroxisomal localization of various small GTPases. By the use of specific antibodies, evidence was provided that except for RhoA, Arf1, Arf6 and Rab11, Rab5 and Sar1 may not localize to peroxisomes. So far, Rab11 has been proposed to play a role in the endocytic transport of cholesterol to the plasma membrane. Therefore, the finding that Rab11 may be implicated in regulating peroxisomal functions is an interesting aspect pointing to the central role of peroxisomes in cellular lipid metabolism.