Isolation of Intact Organelles by Differential Centrifugation of Digitonin-Treated Hepatocytes Using a Table Eppendorf Centrifuge

Miguel Bronfman, Gloria Loyola, and Cecilia S. Koenig

Abstract

A quick subcellular fractionation procedure using differential centrifugation, which is applicable to isolated and cultured cells, is presented. This technique was developed for studying the subcellular localization of phosphorylated proteins in isolated liver cells after various stimuli, but is also applicable to many other situations. The main difference with the usual techniques is that by including digitonin in the homogenization buffer, the procedure is greatly shortened. Furthermore, because the soluble fraction is separated from the particulate fraction very early in the fractionation procedure, subcellular organelles are not exposed to phosphatases and other soluble enzymes such as esterases and proteases during the fractionation. The entire procedure is carried out in an Eppendorf centrifuge, which allows isolation of the cytosolic fraction in less than 1 min, a washed nuclear fraction in about 4 min, a mitochondrial fraction in less than 10 min, and a washed light mitochondrial L fraction in about 40 min. Judging by the behavior of marker enzymes and the morphology of the fractions, the method is highly comparable to classical procedures.